Rusts of pine

Y. Hiratsuka, J.K. Samoil, P.V. Blenis,
P.E. Crane and B.L. Laishley, editors
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RUSTS of PINE Working Party Conference
IUFRO
1989
Banff, Alberta, Canada

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Participants in the IUFRO Rusts of Pine Conference, Banff, Canada, September 18-22, 1989.

Cover photo:

Year-old cones ("Bingham buds") of *pinus monticola* (U.S. Forest Service photo).

RUSTS OF PINE

Proceedings of the
IUFRO Rusts of Pine Working Party Conference
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ABSTRACT

The Third International IUFRO "Rusts of Pine" Working Party Conference was held on September 18–22, 1989, in Banff, Alberta, with participants from nine countries. Fifty-five papers were presented on the distribution, history, taxonomy, histopathology, epidemiology, genetics, disease resistance, and management and control of rust disease of both hard and soft pines.

RÉSUMÉ

La Troisième Conférence du Groupe de travail de l'IUFRO sur les rocailles du pin a eu lieu du 8 au 22 septembre 1989 à Banff, en Alberta, et réunissait des représentants de neuf pays. Un total de 55 communications ont été présentées sur la répartition, l'historique, la taxinomie, l'histopathologie, l'épidémiologie, la génétique, la résistance aux maladies ainsi que sur la gestion et répression de la rouille affectant les pins durs et les pins tendres.

PREFACE

For many years there has been a working party within IUFRO covering research on white pine blister rust, with members from Asia, Europe, and North America. Until 1979, however, forest pathologists interested in rusts of hard pines did not have a similar forum for their research activities. The first IUFRO meeting dealing with rusts of hard pines took place in Florence, Italy, on September 5-7, 1979. The chairman for this conference was sponsored jointly by IUFRO and the National Research Council of Italy. Over 20 pathologists and geneticists from Italy, Austria, Poland, and the United States attended the meeting. The meeting in Florence was preceded by a field tour of forest disease problems in several areas of Austria, led by Dr. Edwin Donaubauer and his staff from the Institute for Forstschutz in Vienna, Austria.

The second meeting of the rusts of hard pines working party was held in Athens, Georgia, USA, on October 1-6, 1984. The Chairman was Dr. H.R. Powers, and the local arrangements were directed by Dr. L. David Dwinell, Dr. Jane Barrows-Broaddus, Dr. E. George Kuhlman, and Roger Belanger. Forty-two pathologists, geneticists, and silviculturalists from Austria, Italy, Canada, Japan, and the United States attended this conference, which was followed by a two-day field trip to research and study sites within the state of Georgia.

During the week of September 16-21, 1985, a combined meeting of the IUFRO working parties on forest gall midges (S2.07-08) and rusts of pines (S2.06.10) was held in Seoul, Korea. Drs. Yong-Joon La and Ke-Ho Ko were cochairmen of the conference. This meeting brought together researchers from Korea, Japan, Austria, Switzerland, and the United States who worked on gall midges, white pine blister rust, and hard pine blister rusts. The meeting was followed by a two-day tour throughout central and southern Korea.

Since the meeting in Seoul included rust research on both hard and soft pines, the organizing committee for the next meeting in Banff, Canada, decided to continue the practice of including rusts of all pine hosts for the Banff conference. This conference was held in the Banff Center, Banff, Alberta, Canada, September 18-22, 1989. Dr. Y. Hiratsuka served as local arrangements chairman, Dr. Peter Blenis, Paul Maruyama, and Pat Crane as the organizing committee, and Dr. Powers as working party chairman. This was one of the largest meetings to date, with 65 pathologists, geneticists, and silviculturalists from India, Canada, the United States, Italy, Japan, People's Republic of China, Sweden, Scotland, and Poland in attendance. During this meeting there was also a one-day field trip to look at forest disease problems in the beautiful mountain areas of Alberta. All of the participants felt that the conference was both informative and enjoyable, and all agreed that it was very effective to have researchers involved in all of the rusts of pines taking part in the conference. At the conclusion of this meeting, Dr. Powers turned over chairman of the working party S2.06-10 to Dr. George Kuhlman.

The time of the next meeting is to be in 5 years, and the location has tentatively been set for Japan. All in all, the progress that we have made over the 10 years since conducting the first meeting in Florence has been very impressive. The conferences have greatly facilitated the exchange of ideas, methods, plant material, and scientists. The fact that we now have a joint forum for researchers covering all aspects of rusts of pines is certain to be very beneficial for future productivity in this area.

H.R. Powers, Jr.
Chairman, IUFRO Rusts of Pine Working Party

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TAXONOMY, PHYLOGENY, AND COEVOLUTION OF PINES AND THEIR STEM RUSTS

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ABSTRACT

We review and reinterpret major events in the evolution of pines and their stem rusts using information from their taxonomy, genetics, biogeography, and fossil history. Understanding of pine evolution has been significantly revised in the last 20 years. Pines appear to have evolved early in the Mesozoic and to have diversified and migrated throughout middle latitudes in the Northern Hemisphere supercontinent, Laurasia, by the end of the Mesozoic. By this time, the major subgenera had appeared (subgenus *Strobus*, oldest) as well as several early subsections, probably including at least *Cembroides, Gerardianae, Strobi, Canarienses, Pineae, Sylvestres, Ponderosae*, and *Australes*. In the early Tertiary, global climates changed and favored the spread throughout middle latitudes of angiosperm floras adapted to hot, humid conditions. These apparently fragmented and displaced pines into local cool dry refugia in polar latitudes, warm dry refugia at low latitudes, and scattered upland refugia at middle latitudes (e.g., present Rocky Mountains and Japan). Several subsections were split north and south. Some of these areas experienced intensive mountain-building, which created environmental heterogeneity that favored pine radiation and created secondary centers of origin (e.g., Mexico). Following the climatic deterioration at the end of the Eocene, tropical-adapted angiosperms were eliminated throughout middle latitudes, and pines replaced them. Radiations of subsections *Oocarpae, Sabinianae*, and *Contortae* and of many species within subsections seem to date to this period.

Rusts appear to be much older than pines, probably arising as autoecious species in the Carboniferous, with ferns and mosses as original hosts. Heteroecism evolved early, enabling rusts to remain specialized on primary hosts but to radiate on evolutionarily more advanced secondary hosts. The conifers were the first new host group, followed by primitive, then more advanced, angiosperm hosts. Estimated trends in angiosperm evolution and approximate dates of origins of pine lineages are used to infer trends in rust phylogeny. Groups of rusts sharing ancient angiosperm hosts also share old pine hosts, including *Cronartium ribicola, C. occidentale, C. quercuum, C. comptoniae*, and *C. flaccidum*. Putatively derived rusts (including secondarily autoecious species and special forms) *C. appalachianum, Peridermium filamentosum*, and *P. yamabense* share primary and alternate hosts that evolved more recently.

Quaternary climate oscillations affected the genetic structure of rusts as well as hosts. Rusts apparently followed their hosts during glacial and interglacial periods. Three distinctive and autoecious white pine rusts occur on nearby mountaintops in Japan and trace their origin to biogeographic events in the Quaternary.

Effective control of pathogens in introduced and endemic situations may depend on an understanding of genetic relationships among both rusts and hosts. Many biochemical tools are available that should be used to clarify some of these relationships.

DEDICATION

We dedicate this paper to the late William B. Critchfield, pine geneticist, systematist, and geographer, whose pioneering work set the stage for future attempts at unraveling the mysteries of pine evolution.

INTRODUCTION

The stem rusts of pine in *Cronartium*, *Peridermium*, and *Endocronartium* are some of the most ancient, ubiquitous, and destructive pathogens of forest trees. Increasing human interference over the past two centuries in these predominantly wild pathosystems has had disruptive and sometimes disastrous consequences. These have resulted from several types of disturbance: from introducing exotic pathogens onto native pines (as with white pine blister rust introduced into North America); from introducing exotic pines into areas of native pathogens (as with eastern white pine (*Pinus strobus*) into Japan); and from upsetting the ecological and genetic balance of endemic pathosystems (as in fusiform rust of southern USA pines). Furthermore, there are suggestions that pine-breeding programs, especially those that involve interspecific hybridization, may be creating bridges for new genetic combinations of native rusts of wider virulence.

If we are to mitigate the deleterious effects of human interference in pine-rust pathosystems and mount effective programs of rust resistance, we must understand the genetic structure of both hosts and pathogens. Information about the phylogenetic and taxonomic relationships of pines and rusts might eventually enable us to gauge the genetic amplitude of the different pathogens, to calculate the risk to domesticated forest crops, and to identify centers of origin for resistance genes. Leppik's (1959) cogent statement still applies: "...our most effective control measure, the breeding of rust-resistant varieties, depends ultimately upon our knowledge of the genetic constitution and phylogenetic relationships of the rusts involved" to which we add, most emphatically, their wild hosts as well. To this end, we review the taxonomy and phylogeny of pines and rusts and describe processes by which pine-rust pathosystems may have coevolved.

PINES

Classification and Taxonomy of Pines

History of Classification

Pines have been described and classified since Classical times. Theophrastus (370-285 B.C.), a pupil of Aristotle, wrote extensively about morphology, reproduction, and uses of pines in his *Enquiry into Plants* (Hort 1916; Abbe 1965; Mirov 1967). Using the Greek names *pitys* and *peuke*, Theophrastus described five Mediterranean pines that can be referred to as modern species. Subsequent Classical geographers and natural historians used Theophrastus' identifications and often mentioned these pines in their writings. Virgil (70-19 B.C.) first used the name *Pinus* to refer to pines in his two lyrical works, *Georgics* and *Eclogues* (Abbe 1965).

Although the name *Pinus* was quickly adopted, its circumscription varied, even into the time of the Linnean systematists. Tournefourt (1694) defined the genus narrowly, whereas Linnaeus (1753) included modern *Cedrus*, *Larix*, *Picea*, and *Abies* as well as pine in *Pinus*. The genus was quickly reduced again to Tournefourt's framework by Miller in 1754 (in Little and Critchfield 1969), who segregated *Abies* and *Larix*. *Cedrus* and *Picea* were soon also removed, and the narrow definition of *Pinus* was widely accepted by the mid nineteenth century. By that time, pines were distinguished from other conifers by needle like leaves that were borne on short shoots in fascicles of two to five and that had a sheath of bud scales at the base of the fascicle (Spach 1842; Endlicher 1847; Carriere 1867).

Since the time of Linnaeus, over 40 serious systematic treatments of *Pinus* have appeared (reviewed in Mirov 1967; Little and Critchfield 1969; Price 1989). Many of these have relied on phenetic similarities of single traits or trait complexes (e.g., needle characteristics, Koehne 1893; Doak 1935; Jaehrig 1962; de Ferre 1965; cone characteristics, Klaus 1980, 1989; wood anatomy, Hudson 1960; van der Burgh 1973). In the last two decades, systematists have brought new biochemical and molecular tools to the study of pine classification, including analyses of terpenes (Mirov 1967), immunoglobins (Price et al. 1987), and DNA (Strauss and Doerksen 1990; Kossack 1989).

The most useful classifications are holistic and attempt to summarize and reconcile information from many types of traits. An evolutionary framework is implicit in ordering groups, and these systems are based on hypotheses about phylogenetic relationships and evolutionary transformations. George Russell Shaw (1914) was the first to apply this type of analysis to pines in his landmark synthesis, *The Genus Pinus*. Shaw used information on morphology of the shoots, leaves, ovulate and pollen cones, seeds, wood anatomy, bark, growth habit, habitat, and world distribution to classify the pines. Shaw divided the pines into two widely accepted sections, *Haploxylon* and *Diploxylon* (as named by Koehne 1893), based on the presence of one or two vascular bundles in the needle. He further distinguished four subsections and 13 unnamed groups.

Subsequently, several evolutionary systematists have modified Shaw's system. Pilger (1926) elevated *Haploxylon* and *Diploxylon* to subgeneric status and rearranged the ordering of some groups of *Diploxylon* pines. Pilger's classification was criticized for its overreliance on needle number (Duffield 1952; Mirov 1967), and Shaw's ordering of the groups was more widely retained. A major contribution to evolutionary ordering of species and higher taxa, especially within subgenus *Diploxylon*, came from the extensive artificial hybridization work of Duffield (1952). This information corroborated Shaw's system in many places but also indicated new groupings in several places. A French group of systematists (de Ferre 1965; Campo-Duplan 1950; Flous 1937; Gaussen 1960) studying morphology of juvenile forms, resin duct position, and especially pollen anatomy proposed a classification of pines that differed radically from Shaw's. The systematic interpretations of this group have not been widely accepted because they were not consistent with other kinds of data.

Genus **Pinus**: *Little and Critchfield (1969)*

The modern authority, following Shaw, is the classification of Little and Critchfield (Critchfield and Little 1966; Little and Critchfield 1969)[1]. Although retaining much of Shaw's reasoning and order, Little and Critchfield incorporated new types of systematic information based on genetic data, especially from contemporary studies on hybridization and biochemical variation. They also brought the nomenclature up to international code (Lanjouw 1966). Although there have been slight modifications and additions proposed subsequently (especially in little known groups such as the Mexican pines; see next section), Little and Critchfield's system is so widely accepted that we summarize it here intact except for a few recent updates.

---

[1] We adopt the Little and Critchfield (1969) system and refer authority of pine names to them, although pines not included in their system are referenced.

Little and Critchfield (1969) divided *Pinus* into 3 subgenera, 4 sections, 15 subsections, and 94 species (Fig. 1). *P. longaeva* Bailey (1970) was not segregated by Little and Critchfield. The species occur throughout the Northern Hemisphere (one species crosses the Equator at Sumatra) in temperate regions and tropical mountains. Little and Critchfield added subgenus *Ducampopinus* with only a single species, *P. krempfii* of Vietnam, distinct from all other pines in its flattened leaves and absence of ray tracheids. Subgenus *Strobus* (= Shaw's *Haploxylon*) comprises 2 sections, 5 subsections, and 31 species distributed in North America and Eurasia; subgenus *Pinus* (= Shaw's *Diploxylon*) comprises 2 sections, 9 subsections, and 62 species (Fig. 1). Maps are in Little and Critchfield (1969). The two major subgenera are characterized by the following traits, with some exceptions:

	Pinus subgenus	
	Strobus	*Pinus*
Vascular bundle	Single	Double
Fascicle sheath	Deciduous	Persistent
Base of fascicle bracts	Not decurrent	Decurrent
Needles/fascicle	1-5	2-5
Staminate buds	Not preformed	Preformed
Spring shoots	Uninodal	Uni- or multinodal
Ray tracheids	Smooth walls	Dentate walls
Annual rings	Obscure	Distinct
Umbos on cone scales	Terminal and dorsal	Dorsal

The two major subgenera were further divided into two sections each. Within subgenus *Strobus*, section *Strobus* has two subsections with 19 species of mostly northern distribution in North America and Eurasia. Section *Parrya* has three subsections with 12 species: one distributed in central Asia, one in southwestern USA, and one in southwestern USA and Mexico. The following traits distinguish the sections:

	Subg. *Strobus*, sect.	
	Strobus	*Parrya*
Umbos on cone scales	Terminal	Dorsal
Needles per fascicle	5	1-5
Needle epidermis and hypodermis	Distinct	Similar
Conelet scales	Pointed	Rounded
Wood ray cells	Large pits	Small pits

Within subgenus *Pinus*, section *Pinea* is a heterogeneous group with three subsections and five species of mostly southern distribution in Mexico, the Mediterranean region, and central Asia. The section is not distinguished by a consistent set of traits from the other section in the subgenus. Rather, each subsection within *Pinea* has a unique set of subgenus *Strobus* traits that sets it apart within subgenus *Pinus* and lacks advanced traits found in section *Pinus*. Section *Pinus* is the largest in the genus, comprising six subsections in North America and Eurasia with 57 species distributed mostly in the south but a few far north. Distinguishing traits include:

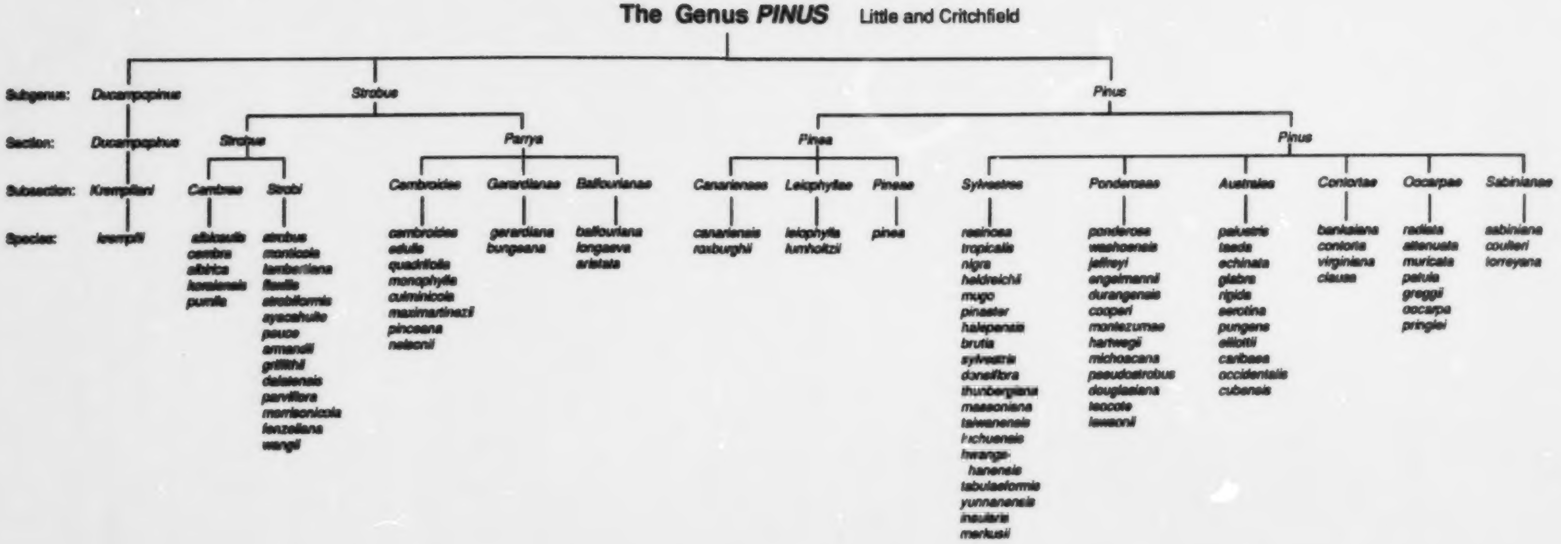
The Genus PINUS Little and Critchfield
Subgenus:
Section:
Subsection:
Species:
Ducampopinus
Ducampopinus
Krempfiani
krempfii
Strobus
Strobus
Parrya
Cembrae
albicaulis
cembra
sibirica
koraiensis
pumila
Strobi
strobus
monticola
lambertiana
flexilis
strobiformis
ayacahuite
peuce
armandii
griffithii
dalatensis
parviflora
morrisonicola
fenzeliana
wangii
Cembroides
cembroides
edulis
quadrifolia
monophylla
culminicola
maximartinezii
pinceana
nelsonii
Gerardianae
gerardiana
bungeana
Balfourianae
balfouriana
longaeva
aristata
Pinus
Pinea
Pinus
Canarienses
canariensis
roxburghii
Leiophyllae
leiophylla
lumholtzii
Pineae
pinea
Sylvestres
resinosa
tropicalis
nigra
heldreichii
mugo
pinaster
halepensis
brutia
sylvestris
densiflora
thunbergiana
massoniana
taiwanensis
luchuensis
hwang-
hanensis
tabulaeformis
yunnanensis
insularis
merkusii
Ponderosae
ponderosa
washoensis
jeffreyi
engelmannii
durangensis
cooperi
montezumae
hartwegii
michoacana
pseudostrobus
douglasiana
teocote
lawsonii
Australes
palustris
taeda
echinata
glabra
rigida
serotina
pungens
elliottii
caribaea
occidentalis
cubensis
Contortae
banksiana
contorta
virginiana
clausa
Oocarpae
radiata
attenuata
muricata
patula
greggii
oocarpa
pringlei
Sabinianae
sabiniana
coulteri
torreyana

Figure 1. Taxonomy of the genus *Pinus* according to Little and Critchfield (1969), showing the species classed into subgenera, sections, and subsections.

	Subg. *Pinus* sec.	
	Pinea	*Pinus*
Subg. *Strobus* traits	Present	Absent
Seed wing long, dehiscent	Absent	Present
Spring shoots	Uninodal	Uni- or multinodal

Little and Critchfield further subdivided each section into subsections. Although they followed Shaw's subdivisions in many cases, they used evidence from modern studies on genetic relationships and hybridization to develop the subsections and classify species in subsections. In general, species within a subsection are able to hybridize, whereas crossability is reduced among species of different subsections (Critchfield 1975). This resulted in revisions, especially in section *Pinus*, where Shaw's reliance on cone morphology led to some uncorroborated classifications. Three subsections have bihemispheric distributions; the others are limited to North America or Eurasia.

Within subgenus *Strobus*, section *Strobus* is divided into two subsections (Fig. 1). Subsection *Cembrae*, the stone pines, comprises five species of high latitudes and high altitudes, four of which are in Eurasia and one is in western North America. *Cembrae* pines have cones and seeds uniquely adapted to disperal by alpine birds of the genus *Nucifraga*, which is the primary basis for their taxonomic segregation. The 14 species of subsection *Strobi*, the white pines, are distributed in northern Eurasia (8 species) and North America (6 species). The subsections are characterized by

	Subg. *Strobus*, sect. *Strobus* subsect.	
	Cembrae	*Strobi*
Cone dehiscence at maturity	Indehiscent	Dehiscent
Seeds	Wingless	Winged

Section *Parrya* within subgenus *Strobus* comprises three subsections (Fig. 1). Subsection *Gerardianae* has two species in south and east Asia; subsection *Balfourianae*, the foxtail pines, has two species (later divided into three species) restricted to high elevations in southwestern USA; and subsection *Cembroides*, the pinyon or nut pines, has eight species limited to semiarid regions of southwestern USA and Mexico. Plant exploration and genetic analyses of the little-known pines of Mexico continue to revise subsection *Cembroides*. Distinguishing traits of these subsections are

	Subg. *Strobus*, sect. *Parrya* subsect.		
	Gerardianae	*Balfourianae*	*Cembroides*
Needles per fascicle	3	5	1-5
Seeds	Large, rudimentary wing	Small, winged	Large, wingless

Each of the three subsections within section *Pinea* of subgenus *Pinus* (Fig. 1) is distinguished by unique retention of certain subgenus *Strobus* traits. Subsection *Pineae* is monotypic, containing *P. pinea* of the Mediterranean. Subsection *Canarienses* comprises two highly disjunct species, one of the

Canary Islands off northwest Africa and one of the Himalayas, and subsection *Leiophyllae* comprises two Mexican species. The subsections are characterized by

	Subg. *Pinus*, sect. *Pinea* subsect.		
	Pineae	*Canarienses*	*Leiophyllae*
Strobus traits	Seed with rudimentary wing (cf. *Gerardianae*)	Seed with undetachable long wing (cf. *Strobi*)	Leaf sheath deciduous (cf. *Strobus*)
Needles/fascicle	2	3	3-5

The six subsections of section *Pinus*, subgenus *Pinus*, contain 60% of the species in the genus and are distributed in nearly every pine habitat. *Sylvestres* is the largest subsection, containing 19 species--all in the Old World except *P. resinosa* and *P. tropicalis* of eastern North America. The remaining subsections are restricted to North America. Subsection *Contortae* comprises FOUR species distributed throughout northern and eastern North America. The 11 species of subsection *Australes*, the southern yellow pines, occur mostly in southeastern USA, with some on the Caribbean islands and adjacent Central America. Subsection *Ponderosae*, the western yellow pines, has 13 species in western USA, Mexico, and Central America; subsection *Oocarpae* has seven species in southwestern USA, Mexico, and Central America; and subsection *Sabinianae* has 3 species in southwestern USA. The subsections are distinguished by unique combinations of traits rather than single diagnostic characteristics.

Subg. *Pinus*, sect. *Pinus*, subsection:	
Sylvestres	Small symmetrical cones that open at maturity, prickle on cone scale
Ponderosae	Moderate to large symmetrical cones that open at maturity, prickle on cone scale, cones leave basal scales on branch
Australes	Symmetrical cones that open at maturity, prickle on cone scale, spring shoots multinodal
Sabinianae	Massive symmetrical cones with slow opening past maturity, cone scales terminate in long stout beak, seeds large, with thickened base of wing
Contortae	Asymmetrical cones that remain closed at maturity, two needles per fascicle, needles < 8 cm
Oocarpae	Asymmetrical cones that remain closed at maturity, mostly three needles per fascicle, needles > 8 cm

Recent Revisions and Unresolved Questions

Several areas of pine classification remain ambiguous, either because of confusing patterns of relationships or because the pines are little known. In the first category is the uncertain position of *P. krempfii*. The species is distinguished from all other pines by its flattened leaves and absence of ray tracheids. Discovered and described in 1921 (LeComte 1921), it has been placed in subgenus *Strobus* (then *Haploxylon*) (Pilger 1926), in a new subgenus *Ducampopinus* (Gaussen 1960), and elevated to an independent monotypic genus *Ducampopinus* (Chevalier 1944; Buchholz 1951; de Ferre 1953). It is more commonly accepted to be in the genus *Pinus*, although whether it is a separate subgenus (e.g., Little and Critchfield 1969) or within *Strobus* (Florin 1931; Erdtman et al. 1966) is not clear. Despite its differences, *P. krempfii* has many subgenus *Strobus* (and especially section *Parrya*) traits--including single vascular bundle, deciduous needle sheath, base of fascicle bracts not decurrent, 2 needles per fascicle, cones with thin scales, dorsal umbos, and thin prickle--and wood resembling subgenus *Strobus*. Unfortunately, living tissue is extremely difficult to obtain, and none of the modern biochemical and molecular investigations have included this species.

Another species of uncertain affinity is the recently described *P. rzedowskii* Madrigal and Caballero (1969). A rare species limited to three populations in Michoacan, Mexico, it was originally placed in subsection *Balfourianae*. The species is haploxylon, has dorsal umbos on the cone scales (thus placing it in *Parrya*), has many *Cembroides* features, yet has an articulate seed wing found only in section *Pinus* and in *P. aristata* of subsection *Balfourianae*, and has several features found only in *P. canariensis* of subsection *Canarienses*. Klaus (1989) felt its combination of characteristics was distinct enough to warrant a new monotypic subsection *Rzedowskiae* within section *Parrya*.

There are several ambiguous areas of classification in section *Strobus*, subgenus *Strobus*. A general question is whether subsection *Cembrae* is a natural or polyphyletic group. Shaw (1914) segregated *Cembrae* from *Strobi* on the assumption that retention of seeds in the indehiscent cone (*Cembrae*) and dispersal of seeds by birds are monophyletic traits. This assumption has been investigated and defended by Lanner (1982, 1988, 1990) and challenged by Critchfield (1986). Critchfield reasoned that although the available evidence is highly discordant, it does not support the monophyletic origin of *Cembrae* traits.

A specific question within section *Strobus* is the relationship of *P. lambertiana*. Critchfield (1986) summarized the evidence for the apparent genetic isolation of *P. lambertiana* from its supposed closest relatives, the other western North American species of *Strobi*. The suggestion that it might not belong in subsection *Strobi* (Wright 1962) was dismissed by Critchfield (1986), but there is no good explanation for its close genetic relationship to Asian *Strobi* pines.

Subsection *Cembroides* has received much recent investigation. In the last two decades, a large amount of literature has accumulated on genetics and systematics of the pinyon pines (summarized in Passini et al. 1988; Zavarin 1990). Many new species and species complexes have been described, with as many as 16 species in the subsection. The relationships and ranks of many of these remain uncertain, although there is general agreement that there are three distinct groups within the subsection (Zavarin 1988): *Cembroidae* with most of the species, *Pinceanae* with *P. pinceana* and *P. maximartinezii*, and *Nelsoniae* with *P. nelsonii*.

In subgenus *Pinus*, ambiguities remain in determining the natural classification of subsection *Oocarpae* and its relation to pines in *Ponderosae* and *Australes* (reviewed in Millar 1986). Shaw's classification of these groups, which relied heavily on assumed evolutionary trends in cone serotiny, was significantly revised by later genetic and crossing data (Duffield 1952; Critchfield 1967). These authors recognized that closed cones had evolved independently at least four times among pines and were not reliable indicators of monophyly. Duffield (1952) transferred 9 of the 16 species in Shaw's *Insignes* to other groups, leaving 7 that constituted Critchfield and Little's (1966) subsection *Oocarpae*.

The *Oocarpae* subsection remains heterogeneous. Although some species cluster into natural groups (e.g., those in California), other members have disparate relationships both within the subsection and to species in other subsections. Barriers to crossing exist among some Latin American and Californian members of *Oocarpae*, while hybridization is possible to one species of *Australes* and two species of *Ponderosae* (Critchfield 1967).

Several new classification systems for the genus have appeared since Little and Critchfield (1969). Van der Burgh (1973, with modifications in Farjon 1984) emphasized wood anatomy along with other morphological characteristics. He did not split the system into the traditional subgenera but recognized instead eight sections (two with haploxylon pines and six with diploxylon) with 15 subsections. In his figure (in Farjon 1984), he implies that the diploxylon pines arose independently from primitive haploxylon pines. Although many of the subsections are similar to Little and Critchfield, in some where they differ, van der Burgh's system does not conform to patterns of hybridization (Millar 1986).

Klaus (1980, 1989) developed an evolutionary classification that relies heavily on cone morphology of fossil and extant species, although he incorporated some other morphological features. His system borrows elements from both Little and Critchfield (1969) and van der Burgh (1973, in Farjon 1984) and has the two traditional subgenera, *Strobus* and *Pinus*, seven sections, and 13 subsections. The main changes are in the addition of a new subsection in section *Parrya*, *Rzedowskiae*, and the segregation of *P. resinosa* into a new subsection, *Resinosae*, in section *Pinus* (Klaus 1989).

Analyses of DNA polymorphisms (Strauss and Doerksen 1990; Kossack 1989) are consistent with the subgeneric and section divisions of Little and Critchfield (1969). Haploxylon and diploxylon pines are unambiguously distinct, with the two subgenera robustly monophyletic in their analyses. DNA analyses resolved relationships best at the level of subgenus through distinct subsections, whereas subsections within section *Strobi* and within section *Pinus* were not clearly separated.

Phylogeny and Evolution of Pines

The classification systems described above provide hypotheses about evolution by suggesting common origins for sets of species. They are static models, however, in that they do not indicate the complexity of evolutionary time, phylogenetic linkages, degree of relationships, evolutionary paths, or evolutionary trends. Our goal here is to provide a historical picture of pines as they evolved from a pre-*Pinus* ancestor to their modern diversity. The scenario we develop is speculative yet synthetic in that we attempt to integrate different kinds of evidence and build on recent models of others (e.g., Miller 1976; Axelrod 1986). When evidence was contradictory, we chose the most parsimonious solution.

There are three main lines of evidence useful for interpreting the history of pines. The first is genetic relationships among extant species. Degree of relationship is inferred from comparative studies of anatomy, morphology, physiology, biochemistry, and molecular biology, and from studies of natural and artificial hybridization. The fossil record is a second source. Although there are biases in what gets preserved and errors made in identification and interpretation, fossils provide direct evidence and dates for the occurrence of specific morphologies in specific locations and indirect evidence for reconstructing paleoclimates. A third source is biogeography. Present habitats and distributions of extant species as well as regional paleogeography and plate tectonics provide important clues to evolutionary history.

Origins of the Genus Pinus

Interpretation of the early evolution of the genus *Pinus* and the family *Pinaceae* has been significantly revised by comparative analyses of internal cone anatomy in fossil and extant species (Miller

1976, 1977, 1982, 1988; Stockey 1984). These studies identified four fixed traits of internal cone anatomy that distinguish pines from all other extant and fossil pinaceous genera (Miller 1976). A surprising result was that fossil cones having external anatomy of pines did not necessarily have the diagnostic internal pine characteristics. This was especially true of pinaceous remains from the Mesozoic (see Table 1 for geologic times), many of which had been previously identified as pines. Most of these were reclassified into two extinct pinaceous genera, *Pityostrobus* (Nathorst) Dutt (1916) (a highly diverse taxon, probably comprising several natural genera) and *Pseudoaraucaria* (Alvin 1957, 1960; Miller 1976). *Pityostrobus* is now recognized as the pool of variation out of which *Pinus* and other modern genera of the pine family evolved (Fig. 2).

Two further revisions followed from the studies of internal anatomy of fossils. One is that none of the modern pinaceous genera is as old as previously thought, with most genera having no or few

Table 1. Approximate ages and durations of geologic times in the history of the earth

ERA	Period	Epoch	Duration (millions of years)	Millions of years ago
Cenozoic	Quaternary	Holocene	Approximately the last 10 000 years	
		Pleistocene	2.4	2.5
	Tertiary	Pliocene	4.5	7
		Miocene	19.0	26
		Oligocene	12.0	38
		Eocene	16.0	54
		Paleocene	11.0	65
Mesozoic	Cretaceous		71	136
	Jurassic		54	190
	Triassic		35	225
Paleozoic	Permian		55	280
	Carboniferous		65	345
	Devonian		50	395
	Silurian		35	430
	Ordovician		70	500
	Cambrian		70	570
Precambrian			4030	4500

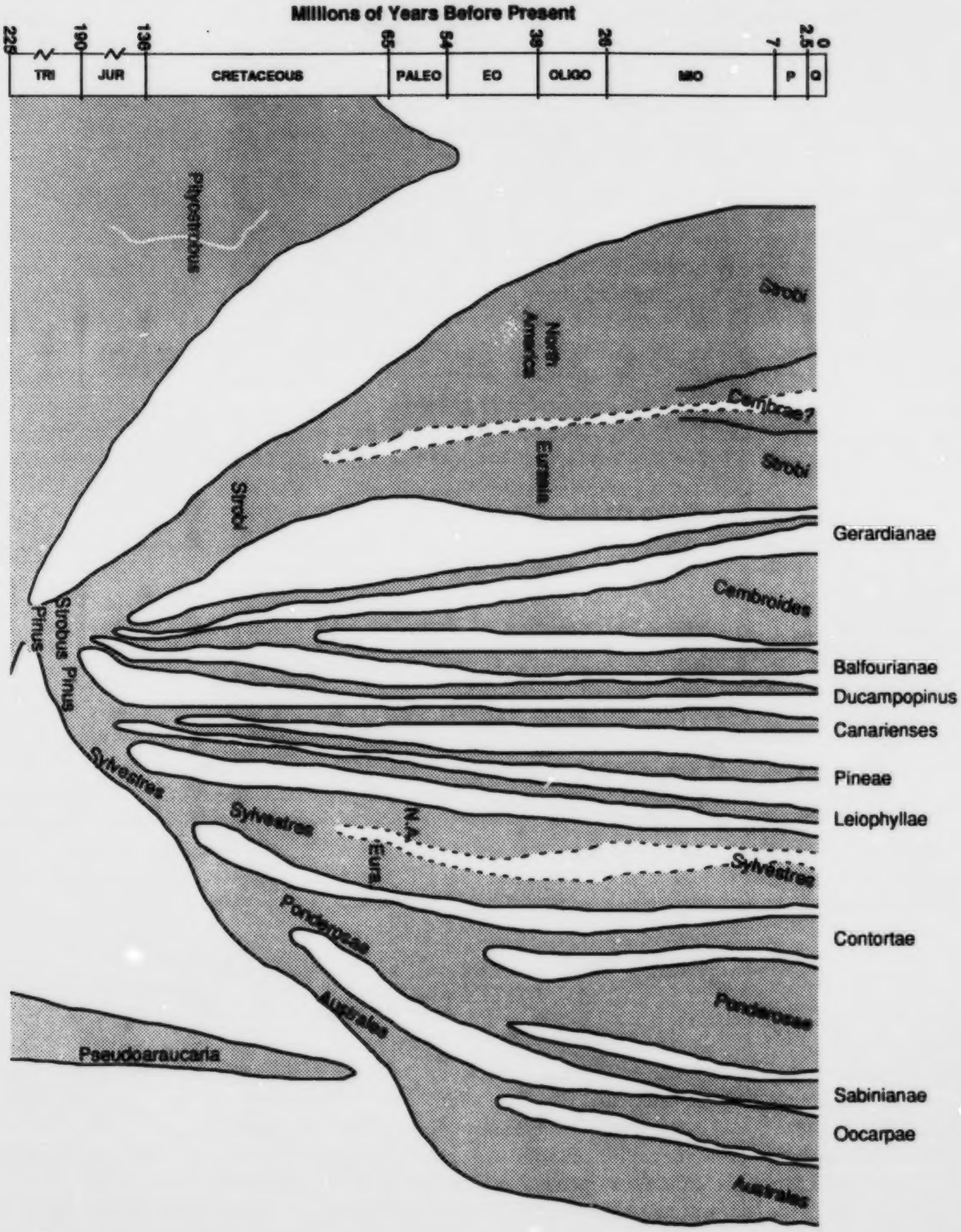
Millions of Years Before Present
225
190
136
65
54
38
26
7
2.5
0
TRI
JUR
CRETACEOUS
PALEO
EO
OLIGO
MIO
P
Q
Pityostrobus
Strobi
North America
Cembrae?
Strobi
Eurasia
Strobi
Gerardianae
Cembroides
Strobus
Pinus
Pinus
Balfourianae
Ducampopinus
Canarienses
Pineae
Leiophyllae
Sylvestres
Sylvestres
N.A.
Eura.
Sylvestres
Contortae
Ponderosae
Ponderosae
Australes
Sabinianae
Oocarpae
Australes
Pseudoaraucaria

Figure 2. Hypothesized phylogeny of the pines, showing their origins from *Pityostrobus* in the Mesozoic and their divergence over geologic time into modern subgenera, sections, and subsections. Subsections that occur in North America and Eurasia are indicated. See Table 1 for geologic times.

remains from the Mesozoic. Previously, pines and other genera in the pine family were believed to have abundant fossil records extending into the Jurassic (summarized in Mirov 1967). The only modern pinaceous genus with a definite Mesozoic record is *Pinus*, which is known from a single species, *P. belgica*, found in Early Cretaceous (130 million years ago) sediments in Belgium (Alvin 1960) and from many species found in sediments of mid and late Cretaceous (Penny 1947; Miller 1976; Robison 1977; Blackwell 1984; Miller and Malinky 1986; Stockey and Ueda 1986; Stockey and Nishida 1986). *Pinus* is now considered to be the oldest genus in the pine family, whereas previously (Mirov 1967) it was thought to be the youngest.

The diversity of forms already present in Cretaceous pine fossils indicates that pines arose even earlier, in the Triassic or Jurassic (Miller 1976; Eguiluz Piedra 1985; Axelrod 1986) (Fig. 2). There is still mixed opinion as to whether the first pines were subgenus *Strobus* or *Pinus*. Early morphologists (Shaw 1914; Doak 1935) thought subgenus *Strobus* had many primitive traits relative to *Pinus* and shared by *Pityostrobus* and other modern pinaceous genera. These include high number of needles per fascicle, resin canal placement, cotyledon anatomy, thin cone scales, terminal umbos on the cone scales, cylindrical symmetric cones, primitive seed wings, uninodal spring shoots, small lateral ray pits of the wood, and thick-walled ray parenchyma in the wood. Contradicting this is the predominance of subgenus *Pinus* in the Cretaceous fossil record (Miller 1976; Axelrod 1986; Miller and Malinky 1986; Stockey and Nishida 1986; Stockey and Ueda 1986). Only fragmentary and questionable fossil evidence exists for subgenus *Strobus* in the Cretaceous (Jeffrey 1908; Stopes and Kershaw 1910; Penny 1947). Several new studies have suggested that pines of section *Parrya*, subgenus *Strobus*, were among the first lineages to evolve (van der Burgh, in Farjon 1984; Klaus 1989; Strauss and Doerksen 1990).

The center of origin of pines remains uncertain. At the beginning of the Mesozoic, the continents were still together in one land mass, Pangaea (Smith et al. 1981). By the early Jurassic, the northern continent, Laurasia, began to drift from the southern continent, Gondwanaland. Since the earliest pinaceous fossils (*Pityostrobus*, *Pseudoaraucaria*, and *Pinus*) were found at temperate middle paleolatitudes (Eguiluz Piedra 1985; Axelrod 1986) in eastern North America and western Europe, it is tempting but not conclusive to suggest that pines originated there. Regardless of where they arose, it is clear that pines spread rapidly at temperate paleolatitudes across Laurasia in the early and mid Mesozoic (Eguiluz Piedra 1985). By the Cretaceous, *Pityostrobus* and *Pinus* had reached both eastern and western extremes of the continent (California: Miller 1976; Japan: Stockey and Ueda 1986; Stockey and Nishida 1986) as well as many inland locations. These early migrations must have been accompanied by major genetic radiations, with subgenus *Pinus* diverging early and, like subgenus *Strobus*, spreading rapidly across Laurasia.

Cretaceous Evolution of Pines

The Cretaceous was a time of major radiation in *Pinus* during which all the sections and many subsections appear to have originated (Axelrod 1986). Compared with the present, the climate of the Cretaceous appears to have been warmer and drier, with seasonality at middle latitudes, although less latitudinal zonation and little topographic variability (Savin 1977; Hallum 1984; Axelrod 1986; Parrish 1987). These conditions were ideal for dispersal of pines. The breakup of Laurasia into North America and Europe (Smith et al. 1981), which began in the late Cretaceous (Fig. 3), created a barrier to migration that can be used to date the groups that now exist in both continents. Although there were transient connections between North America and Europe later during the Tertiary and Quaternary (discussed below), these were unlikely to be major routes of pine migration. Thus, monophyletic groups within the genus with major distributions in both North America and Europe must have evolved prior to the breakup

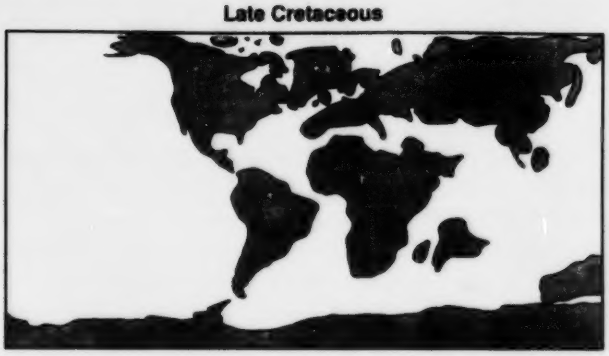

Figure 3. **Generalized map of the continents during the Late Cretaceous.** The supercontinent Pangaea began to break into Laurasia and Gondwanaland in the Jurassic. By the Late Cretaceous, Laurasia was beginning to split into North American and Eurasian continents. The Tethys Sea still formed the southern shore of Eurasia, and India had not yet collided with Eurasia.

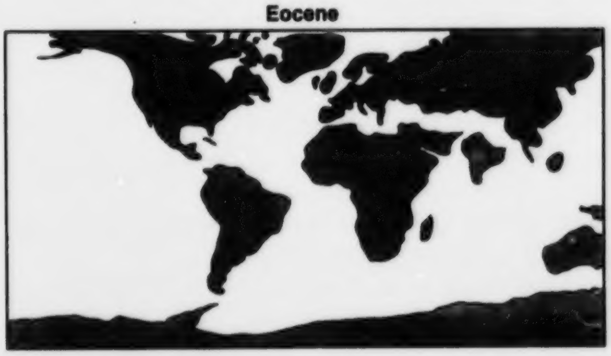

Figure 4. **Generalized map of the continents during the Eocene.** Although the North American and Eurasian continents were mostly isolated, land connections persisted transiently during the Eocene in the North Atlantic and Beringia. The Tethys Sea persisted throughout this epoch.

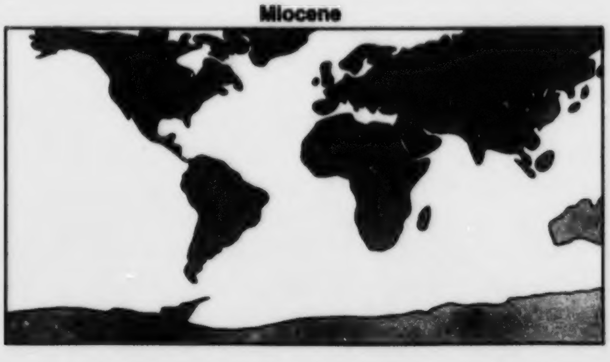

Figure 5. **Generalized map of the continents during the Miocene.** North America and Eurasia have assumed near-modern orientations and latitudes, the North Atlantic connection has been severed, and by the mid Miocene the Beringia connections also were severed.

of Laurasia. This dates the divergence of the two major subgenera prior to the Tertiary, which is corroborated by the existence of Cretaceous fossils of both subgenera. An ancient monophyletic origin for subgenus *Pinus* is supported by molecular data (Strauss and Doerksen 1990; Kossack 1989), and supported by patterns of hybridization (Critchfield 1975) and comparative morphology (Shaw 1914; Little and Critchfield 1969).

Several other lineages appear to have diverged soon after the origin of the subgenera (Fig. 2). Within the unusually diverse section *Parrya* are found traits otherwise only in subgenus *Pinus*, and in the diverse section *Pinea* are found traits otherwise only in subgenus *Strobus*. This suggests early origins for lineages in these sections before the subgenera had evolved other derived traits. Molecular (Strauss and Doerksen 1990; Kossack 1989) and cladistic (van der Burgh, in Farjon 1984) analyses confirm the early divergence times of the subsections within these sections. The little known *P. krempfii*, with its unique combination of traits, may also have arisen as a minor lineage from the early pine gene pool (Fig. 2) either distinct from the other subgenera (as suggested by evidence leading to Little and Critchfield's classification) or allied to subgenus *Strobus* (summarized in Erdtman et al. 1966; van der Burgh, in Farjon 1984). Despite the antiquity suggested for these clades within *Parrya*, *Pinea*, and *Krempfiani*, they either did not radiate early, or, if they did, many of the lineages went extinct without leaving a record. There is little hint of their presence in the known Cretaceous or early Tertiary fossil record, and many of the radiations within the subsections are considered to be recent events (discussed below).

Within each of the two major subgenera was a lineage that migrated widely prior to the end of the Cretaceous and left abundant evidence of early radiations. Pines of subsection *Strobi* and subsection *Sylvestres* probably evolved following the divergences of sections *Parrya* and *Pinea* (Fig. 2). The fact that DNA analyses resolved evolutionary distinctions among subsections within *Parrya* and *Pinea* but not among subsections within *Strobus* or *Pinus* points to the younger ages of the last two sections (Strauss and Doerksen 1990; Kossack 1989). Within their respective sections, *Strobi* and *Sylvestres* are characterized by fewer derived traits than the other subsections (Shaw 1914; Klaus 1980). Distribution of species in the subsections in both North America and Eurasia indicates that they evolved prior to the close of the Mesozoic. Many Cretaceous pine fossils including the oldest known, *P. belgica* (Alvin 1960), have traits that ally them to subsection *Sylvestres* (Penny 1947; Pierce 1957; Chaney 1954; Robison 1977; Stockey and Nishida 1986). They have been found in deposits in western Europe; eastern, central, and western North America; and Japan.

Subsections *Ponderosae* and *Australes* appear to be closely related (Shaw 1914; Duffield 1952; Gaussen 1960; Jaehrig 1962; de Ferre 1965; Klaus 1980). The evolutionary transformations of traits suggested by Shaw (1914) indicate these subsections to be derived from *Sylvestres*, with *Australes* having the most derived traits of the three subsections (e.g., multinodal spring shoots). Fossil cones allied to *Ponderosae* and *Australes* are the next most common pines after *Sylvestres* in the Cretaceous and early Tertiary record. *Ponderosae*-like cones have been found in both western and eastern North America (Penny 1947; Robison 1977; Stockey 1983, 1984), while *Australes*-like cones have been found in eastern North America (Blackwell 1984; Miller and Malinky 1986). During the late Cretaceous, an epicontinental seaway existed in central North America, effectively isolating the eastern and western regions floristically (Muller 1970; Eguiluz Piedra 1985). Consideration of all the evidence together suggests that a lineage of *Sylvestres* had migrated throughout North America by the mid Cretaceous and that *Ponderosae* evolved from this gene pool and also spread throughout North America. *Australes* may have evolved from an eastern lineage of *Ponderosae* or *Sylvestres*, subsequently diverging in isolation from western elements of these groups due to the epicontinental seaway. Extant *Ponderosae* occurs only in western North

America, Mexico, and Central America, while *Sylvestres* occurs only in eastern North America and the Caribbean (Little and Critchfield 1969).

Tertiary Evolution of Pine: Paleocene and Eocene

The transition from Mesozoic to Cenozoic eras (65 million years ago, Table 1) marked the beginning of a major upheaval in pine evolution that lasted 35 million years. It ended in the evolution of most modern subsections and of patterns of speciation within subsections[2] (Axelrod 1986). By the early Cenozoic, the breakup of Laurasia was well under way with North America and Eurasia isolated at all latitudes except for transient connections in the North Atlantic and Beringia (Tiffney 1985a, 1985b). The Cretaceous mid-continental seaway of North America shrunk in size through the early Tertiary: by the end of the Eocene, it existed only as a major bay penetrating the Gulf coast (Tiffney 1985a). The extensive southern border of Eurasia was formed by the Tethys Sea (Fig. 4) (Plaziat 1981) which included regions now along the Mediterranean, Himalayas (India had not yet collided with Asia), and southeast Asia.

Major continental movements were only part of the changes of the early Cenozoic. This period was also marked by dramatic changes in global climate (Wolfe 1975, 1978, 1985; Hsu 1983; Parrish 1987). Relative to the early-middle Cretaceous, the late Paleocene and early Eocene (and at least two other periods during the mid Eocene) had hot, humid, tropical conditions that were highly equable and aseasonal (Wolfe 1975, 1978, 1985; Buchardt 1978; Parrish 1987). The warm, wet periods lasted 5-10 million years each, and average annual temperatures fluctuated 7-10°C between warm and cool periods (Wolfe 1978). This climate extended in broad zones throughout middle latitudes in both Northern and Southern Hemispheres (Wolfe 1975, 1978, 1985). Within these zones, latitudinal changes in climate were much less exaggerated than at present, and tropical-subtropical conditions extended from 25° to 60-70° latitude (Wolfe 1985). The latitudinal gradient that did exist differed from the present pattern. In general, low latitudes were warm and dry, mid latitudes were tropical and subtropical, and high latitudes were cool and dry (Wolfe 1985; Parrish 1987).

This climate had an enormous effect on global floristics. By the early Eocene, mid to high latitudes were dominated by highly diverse angiosperm floras with species adapted to tropical conditions (reviewed in Wolfe 1975, 1978, 1985; Tiffney 1985a, 1985b; Wolfe and Upchurch 1986). Foliage and reproductive structures were as diverse and had similar adaptations (e.g., in leaf and fruit structure and shape) as in present Indomalaysian rain forests (Reid and Chandler 1933; Wolfe 1975; Tiffney 1985a, 1985b). Only a few nonpinaceous gymnosperms that were adapted to warm, humid conditions (e.g., *Taxodium, Glyptostrobus*) occurred in the drier of these floras. This cosmopolitan angiosperm flora has been called the boreotropical flora to indicate its tropical adaptations at boreal latitudes (Wolfe 1975). The greatest diversity of boreotropical fossils has been found in western and eastern North America, eastern Asia, and western Europe. Homogeneities in floristic composition among the regions have been explained by transcontinental migration across land bridges in the North Atlantic and Beringia (Tiffney 1985a, 1985b). During the hot, humid periods of the Eocene, these bridges were climatically and ecologically able to support boreotropical floras.

What happened to the pines during these periods of angiosperm evolution and dominance? In the Cretaceous, the distribution of pines at temperate, dry, middle latitudes resembled modern pine biogeography. By the Paleocene and early Eocene, pines had virtually disappeared from these latitudes; in fact, there are scant pine remains from these periods at all (Wolfe 1987). Their distribution and subsequent evolution can be explained by postulating strategically located Eocene refugia.

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[2] Millar, C.I. Impact of the Eocene on the evolution of pines. (In preparation.)

The major climate changes that favored the evolution and spread of the boreotropical angiosperm flora during the three (or more) hot periods of the Eocene severely reduced the distribution of pines. Pines are ecologically poor competitors for angiosperms under tropical conditions (Bond 1989). Pines that occurred in habitats increasingly more tropical (= mid latitudes) at these times must have suffered widespread population extinctions and fragmentations. Cool-dry or warm-dry areas became refugia for existing pine populations or for populations that were able to migrate into them as global conditions warmed.

Three major regions in the Northern Hemisphere might have been pine refugia during the hot, humid periods of the Eocene: high latitudes, low latitudes, and dry inland areas at middle latitudes. There is no evidence for boreotropical floras above 70° north. Above these latitudes, there is evidence in North America and Eurasia for low diversity, cold and seasonally adapted coniferous-angiosperm flora (Wolfe 1972, 1978; Savile 1972; Kuc 1974; Norris 1982). Much of the area now under the Arctic Ocean may have been land in the early Tertiary (Wolfe 1985) and could have provided pine habitat. Early Tertiary pine pollen has been recovered from the Mackenzie Delta and Banks Island, Canada at 69-73°N. (Norris 1982) and in Spitsbergen, Norway, at 76-80°N. (Schweitzer 1974). The lower inclination postulated for the earth's axis during the Eocene (Wolfe 1978) would have permitted high enough light levels at high polar latitudes for plant growth.

Scattered evidence points to warm-dry regions in low latitudes (Wolfe 1975, 1978, 1985; Parrish 1987). The Mississippi embayment of North America supported dry subtropical angiosperm flora (Wolfe 1978), and there is evidence that elsewhere in the continent conditions were warmer and drier further south. Although parts of the Mexican and Central American isthmuses were transiently under water during the Mesozoic (Kellum 1944; Eguiluz Piedra 1985), they were elevated during the Eocene and may have provided pine habitat, although fossil deposits are almost absent from this period (Martin and Harrell 1957; Eguiluz Piedra 1985).

Low latitudes along the Eurasian Tethys Seaway, from the present Mediterranean basin across to southeastern Asia, may also have provided warm-dry habitats for pine refugia. There is evidence that despite abundant boreotropical floras at mid latitudes, low latitudes in Asia such as Borneo and Madagascar supported dry floras (Guo 1980).

Among the inland areas at middle latitudes that might have supported pines in the Eocene, inland western North America is best documented. An upland region extended from northern Nevada and central Idaho north to British Columbia (Axelrod 1966). Of the abundant early Eocene fossil floras from this region, pines are known from only a few locations and are within this upland region (summarized in Wolfe 1987). More fragmentary evidence suggests that areas of Japan may also have been refugia (Wolfe 1985).

This major fragmentation of pines during the Eocene had a great effect on their subsequent evolution. The restriction of pines to refugia explains the current pattern of north-distributed and south-distributed subsections and related species within subsections. Subsections that might have radiated from southern refugia in North America (Fig. 2) include *Cembroides*, *Leiophyllae*, *Australes*, *Sabinianae*, and *Oocarpae* (more on the last two below); *Contortae* radiated from northern refugia. In Eurasia (Fig. 2), subsections *Canarienses*, *Pineae*, *Gerardianae*, and *Krempfiani* may have been in southern refugia along the Tethys Seaway (recall India was not attached to the continent, and the area of present Nepal was the southern edge of the continent). Some subsections may have been divided north and south by the Eocene boreotropical flora. For instance, in North American *Strobi* (Fig. 2), ancestors of *P. monticola* and *P. strobus* were in northern refugia; ancestors of *P. strobiformis*, *P. ayacahuite*, and *P. chiapensis* (*P. strobus* var. *chiapensis*) were in southern refugia. In Eurasia (Fig. 2), *P. parviflora* lineages were northern, and the other seven Eurasian *Strobi* lineages were southern (along the Tethys). In *Sylvestres*, the ancestral lineage of *P. resinosa* was in northern refugia, of *P. tropicalis* in southern North America refugia; ancestral lineages of *P. sylvestres*, *P. densiflora*, and *P. thunbergiana* were in northern Eurasian refugia, and lineages of the other 14 species were in southern refugia. Similar north-south patterns can be seen in species of *Ponderosae*. Inland areas of mid-latitude western North America may have served as refugia for subsection *Balfourianae*, for ancestral lineages of *P. flexilis* within *Strobi* and of *P. ponderosa*, *P. jeffreyi*, and *P. washoensis* within *Ponderosae*. The relation of these inland areas in western North America to southern refugia in Mexico is unclear.

In addition to the biogeographical effect that the Eocene had on pines and the divergence that arose due to isolation of lineages, some pine refugia had characteristics that actively promoted pine radiation. These areas became secondary centers of pine origin. The primary example is in Mexico and Central America, which is widely recognized as an area of high pine diversity and radiation. The late Eocene and Oligocene were times of enormous tectonic changes in this area (reviewed in Eguiluz Piedra 1985). Three main mountain ranges rose, and there was abundant volcanism associated with the uplifts. These events created a heterogeneity of habitats, microclimates, and soils favorable for pine divergence and speciation. Thus, two events contributed to make this area a center of pine origin and diversity: first, pines of diverse lineages were concentrated into Mexican refugia by global effects during the Eocene, and second, creation of environmental diversity favored speciation of pines in the area. Subsections *Cembroides*, *Oocarpae*, and southern elements of *Ponderosae* were especially affected by these events (Fig. 2). Similar events may have occurred in Japan, as indirect evidence of subsequent pine radiations suggests, but the fossil record of pines in the Eocene of Japan is not as complete and does not indicate as clearly as North America the sequence of events (Wolfe 1985).

Tertiary Evolution of Pines: Oligocene to Pliocene

The warm and humid tropical conditions of the Eocene came to a sudden end with a dramatic climatic deterioration called the terminal Eocene event (Wolfe 1978, 1985). The estimated drop in average global temperature was as much as 13°C, with conditions becoming cold, dry, and seasonal where they had been hot, humid, and aseasonal. Changes in inclination of the earth and patterns of air circulation (Wolfe 1978) led to latitudinal zonation that resembled the present. North America and Eurasia were completely severed in the North Atlantic, and the final Tertiary isolation of the two continents at Beringia occurred in the Miocene (Fig. 5). During the Oligocene, mountain-building of the early North American cordillera, Alps, and Himalayas commenced, and environmental gradients and heterogeneity were much greater than before (Wolfe 1985, Axelrod 1986).

The effect of these physical changes was equally dramatic on plants. In a short time, the boreotropical flora all but disappeared throughout the middle latitudes and was replaced by conifers and deciduous angiosperms. In one fossil sequence where successive chronological intervals are well preserved, the boreotropical flora was completely replaced by conifers in only one million years (Axelrod 1966). The widespread reappearance of conifers can be interpreted to mean that pines migrated from Eocene refugia and once again became widely distributed at middle latitudes.

The Miocene was a period of pine proliferation in the fossil record (Axelrod 1986). Relative to the climate of the terminal Eocene event, temperatures were somewhat warmer, with lower ranges in annual temperature, approaching the general present conditions (Wolfe 1978). Mountain-building was active, creating orographic effects on climate, and elevational gradients. Mediterranean climates developed in some parts of the world. These were conditions that clearly favored pines, although the unusual floristic associates of pines compared with the present suggest that the ecological relationships were still quite different from modern ones. During the Miocene are found fossil pines with clearly modern alliances, and it seems safe to say that the lineages leading to most modern species or closely related species complexes had diverged by the late Miocene. A few subsections with derived traits, such as *Oocarpae* and *Sabinianae*, may have arisen late in the early Tertiary (Eguiluz Piedra 1985). There is little indication of their existence earlier, and they are related genetically to *Ponderosae* and *Australes* (reviewed in Millar 1986; Critchfield 1966) which may have been progenitor groups.

To this point, the evolution of subsection *Cembrae* has not been discussed. Since questions remain about the monophyly of *Cembrae*, the origin and evolution of its species is unclear. If monophyletic (as defended by Lanner 1990), its North American and Eurasian distribution suggests that it evolved before the breakup of Laurasia at the end of the Mesozoic. Alternatively, the single North American species, *P. albicaulis*, may have migrated from northeastern Asia to North America by way of Beringia land connections. That this land connection existed transiently into the Miocene (and in the Quaternary, see below) and was used as a plant corridor for angiosperms has been amply documented (Tiffney 1985a, 1985b). However, its ecological availability and use by pines is less certain. Through the warm periods of the Eocene, the latitudes of the land bridge were within the zone dominated by subtropical flora, and it probably could only have supported pine growth during later periods. If *Cembrae* is not monophyletic, individual species may have evolved independently from *Strobi* at various times. The highly derived morphology of the cone and the co-adaptation to dispersal by *Nucifraga* birds suggest that pines of *Cembrae* are recent lineages. Without further evidence, the question is unresolved.

Another candidate for transcontinental migration by way of Beringia is the lineage leading to *P. lambertiana* in subsection *Strobi*. If *Strobi* had a northern refugium at high latitudes in Asia, then the migrations south following the terminal Eocene event may have provided an opportunity for migration of a *P. lambertiana* lineage into North America. As described in an earlier section, *P. lambertiana* has unexpected genetic relationships to other members of the subsection. Although other geographically related *Strobi* species are able to hybridize, *P. lambertiana* does not cross with any of the western North American species of *Strobi* (Critchfield 1986). It does, however, cross with two Asian species of the subsection. Similarly, the resistance mechanism of *P. lambertiana* to white pine blister rust resembles that of Asian *P. armandii* more than that of other North American *Strobi* (Kinloch 1982). Finally, unlike other pines of the subsection, there is little evidence of *P. lambertiana* in the fossil record until later in the Tertiary (the relationship of *P. delmarensis* (Axelrod 1986) from the Eocene of San Diego to *P. lambertiana* is uncertain). This hypothesis and other explanations for the unusual relationships of *P. lambertiana* still need to be investigated.

The late Miocene and Pliocene had fluctuating climates of cool versus warm average global temperatures. Mountain-building continued and pines continued to flourish, although local distributions still differed from the present. Lineages leading to closely related species such as *P. longaeva--P. aristata*, *P. parviflora--P. pentaphylla--P. himekomatsu* (Saho 1972; Numata 1974), and species complexes in *Cembroides* most likely diverged during this time.

Quaternary Evolution of Pines

To this point, we have described how climate and tectonic change over vast periods of time may have guided major episodes in the evolution of pines, resulting in the patterns of diversity in the subtaxa we see today. The Quaternary Period, which began only about 2.5 million years ago, represents less than 2% of the time elapsed since the first appearance of pines in the fossil record. Yet it presents an opportunity to examine with finer focus the dynamics of population change that lead ultimately to speciation. Two lines of evidence complementary to each other and unique to this period make this possible: existence of fossil pollen and macrofossils that can be identified with contemporary species, and patterns of genetically determined geographic variation documented for the same species (Critchfield 1984).

The Pleistocene was an epoch of global spasms of great ice sheets advancing and retreating in rapid frequency over large areas of continental Europe, North America, and mountain ranges worldwide. Glacial episodes were followed by interglacial periods of shorter duration, of which the present Holocene epoch beginning about 10 000 years ago is but the latest. Paleontologists are uncertain how many times this cycle was repeated, but some estimates range from 16 to 18 (Bowen 1979). Although the changes in average global temperature between the glacial and interglacial periods (5-10°C for middle latitudes, Bowen 1979) were less than that inferred for the terminal Eocene event, the Pleistocene was unique for the rapid frequency of climatic changes. Sea levels lowered during the cold, dry glacials, opening up migrational routes between different continents and between continents and offshore islands through land bridges. For example, during glacial periods the Bering land bridge was transiently elevated, and Japan and Britain were connected to their continental mainlands. The reverse occurred during the interglacial periods.

Miki (1957) may have been the first to recognize and document, in the forests of Japan, two basic patterns that characterized the response of pines to the sudden and frequent oscillations in climate during the Pleistocene. The mountains of Japan, like those of North America, have a basically north-south orientation providing migratory escape routes to lower latitudes during colder climatic periods. During interglacials species adapted to warm, moist climates (Miki's type B: e.g., *P. densiflora, P. thunbergiana*), expanded in the lowlands and climbed in elevation, forcing competitors up to colder, harsher environments. When the climate reversed, cold-adapted species (type C: e.g., *P. koraiensis, P. pumila*) migrated to lower elevations and expanded in valleys, displacing type B species which were then relegated to small, isolated refugia where local environments were mild enough to allow survival until another cycle began. Type A (*P. fujiii, P. protodiphylla*, and *P. trifolia*, Miki 1957) did not have adequate variability to adapt and became extinct. Tsukada (1985) showed more comprehensively the drastic changes in composition of Japanese forests from glacial to interglacial phases. During the last full glacial, boreal conifers (including *P. koraiensis*) occupied most of the lowlands of the entire Japanese archipelago. Now these forests are largely broad-leaved, or mixed with conifers in the north; elsewhere, most of the conifers have been pushed to higher elevations.

The abrupt oscillations of climate and the resulting effects on pine distribution had drastic impacts on the genetic structure of forest populations. In addition to species extinction and reduction in genetic variation, one of the major consequences was reorganization and redistribution of genetic variation (Critchfield 1984, 1985). Populations that were fragmented continued their short-term evolution in isolation. If parts of the population mosaic were subjected to differential selection pressure or genetic drift as a result of going through a bottleneck, gene frequencies would change. When the cycle shifted and population fragments coalesced again, gene exchange resumed, and gene frequencies changed again. Transient races thus appeared and disappeared, in more or less constant flux. This rationale has been used to explain many of the anomalous patterns of variation found in extant conifer populations (e.g., Critchfield 1984, 1985; Kinloch et al. 1986; Millar et al. 1988). The microevolutionary processes of the Quaternary that may be read in the genetic structure of extant species give insight into events that might have preceded major radiations in the past and into incipient radiations of the future.

STEM RUSTS

Host parasite affinities and geographic distribution of pine hosts, their stem rusts in *Cronartium--Peridermium*, and secondary hosts in different dicotyledon families are summarized in Tables 2 and 3. With respect to host-parasite affinities, only endemic pathosystems are considered; we do not attempt to deal with relationships based on artificial inoculation or on behavior of introduced hosts or pathogens. Much of the same information is in both tables, but each emphasizes a different perspective. From the perspective of hosts (Table 2), it is noteworthy that subgen. *Strobusus* the oldest lineage, has only three rusts. Subsection *Strobi*, with 14 species on all Northern Hemisphere continents, has only one (*C. ribicola*)[3]; while three subsections (*Gerardianae*, *Balfourianae*, and *Krempfiani* in subgenus. *Ducampopinus*) have none. Subgenus *Pinus* hosts 80% of the stem rusts, and most of these are in North America. Six subsections host only one rust, while four subsections have five or more.

Like their hosts, the greatest diversity of the rusts is in North America (especially western North America) with 11 taxa, compared with 4 in Asia and 2 in Europe. Some rusts are confined to only one pine host subsection (or even a single species: e.g., *C. appalachianum* and *P. virginiana*), while others attack 3 or 4. Most have only one alternate host family, but *C. flaccidum* has 10. Four are autoecious; except for *P. harknessii*, these have relatively narrow host ranges.

Classification and Taxonomy of Stem Rusts

The pine stem rusts in *Cronartium--Peridermium* comprise about 15 species in *Melampsoraceae*, a small but taxonomically difficult group. In the opening statement to his monograph "The *Peridermium* Species on Pine Stems" Peterson (1967) declared "taxonomic knowledge of *Cronartium* is in a state of disorder." Having completed our own review, we feel this might still serve as an appropriate conclusion, but must acknowledge with gratitude that without that paper and its sequel on *Cronartium* (Peterson 1973) we would not have made this attempt. Our review follows his concept and nomenclature of the genus, modified only by developments since his papers were published.

To the uninitiated, confusion is apparent from the beginning, with three generic names for different members of a single natural group. *Cronartium* designates the sexual stage of heteroecious species on dicot hosts. *Peridermium* is the form genus used for the aecial stage on pine stems when the alternate host is unknown or does not exist. Some of these *Peridermia* were put into a new genus, *Endocronartium*, erected to accommodate those autoecious species considered to have an endo-type sexual cycle with meiosis (Hiratsuka 1969). We will not discuss *Endocronartium* here because interpretation of the cytogenetic data on which it is based is controversial (Epstein and Buurlage 1988; Gibbs et al. 1988) and will be addressed elsewhere in these proceedings (Hiratsuka 1990), and because the controversy is not relevant to our theme. Whatever their sexual behavior is it seems clear that the autoecious *Peridermia* have a polyphyletic origin, deriving by reduction from closely related heteroecious species.

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[3] Authorities for nomenclature in *Cronartium--Peridermium* are in Peterson (1967, 1973) except where indicated otherwise.

Table 2. Pine hosts of stem rusts in *Cronartium--Peridermium* and their geographic distribution

Pine host (Subg./ Sect.-Subsect.)	No. of species	Continent[a]	Stem rust
Ducampopinus	1	A	(None described)
Pinus			
Pinea			
Canarienses	2	E,A	*flaccidum*
Leiophyllae	2	NA	*conigenum*
Pineae	1	E	*flaccidum*
Pinus			
Australes	11	NA	*comandrae, comptoniae, conigenum, quercuum, strobilinum*
Contortae	4	NA	*appalachianum, comandrae, comptoniae, harknessii, quercuum, stalactiforme*
Oocarpae	7	NA	*comandrae, conigenum, harknessii, stalactiforme*
Ponderosae	13	NA	*arizonicum, comandrae, comptoniae, conigenum, filamenotosum, harknessii, stalactiforme*
Sabinianae	3	NA	*harknessii*
Sylvestres	19	A,E,NA	*comandrae, comptoniae, flaccidum pini, quercuum*
Strobus			
Strobus			
Cembrae	5	A,E,NA	*ribicola, yamabense*
Strobi	14	A,E,NA	*ribicola*
Parrya			
Balfourianae	3	NA	(None described)
Cembroides	8	NA	*occidentale*
Gerardianae	2	A	(None described)

[a] A = Asia, E = Europe, NA = North America.

Table 3. Stem rusts in *Cronartium--Peridermium*, their distribution, and their hosts

Rust species	Continent	Dicot host	Pine host (subsect.)
Cronartium			
appalachianum	eNA	*Santalaceae*	*Contortae*
arizonicum	wNA	*Scrophulariaceae*	*Ponderosae*
comandrae	NA	*Santalaceae*	*Australes, Contortae, Oocarpae, Ponderosae*
comptoniae	NA	*Myricaceae*	*Australes, Contortae, Ponderosae, Sylvestres*
conigenum	swNA	*Fagaceae*	*Australes, Leiophyllae, Oocarpae, Ponderosae*
flaccidum	E,A	(10 families)[a]	*Canarienses, Pineae, Sylvestres*
occidentale	swNA	*Saxifragaceae*	*Cembroides*
quercuum	A,NA	*Fagaceae*	*Australes, Contortae, Sylvestres*
ribicola	A	*Saxifragaceae* *Scrophulariaceae*	*Cembrae, Strobi*
stalactiforme[b]	NA	*Scrophulariaceae*	*Contortae, Oocarpae, Ponderosae*
strobilinum	seNA	*Fagaceae*	*Australes*
Peridermium			
filamentosum	wNA	autoecious	*Ponderosae*
harknessii	wNA	autoecious	*Contortae, Oocarpae, Ponderosae, Sabinianae*
pini	E	autoecious	*Sylvestres*
yamabense	A	autoecious	*Cembrae*

[a] *Acanthaceae, Asclepiadeaceae, Balsaminaceae, Gentianaceae, Loasaceae, Paeoniaceae, Scrophulariaceae, Solanaceae, Tropaeolaceae, Verbenaceae.*

[b] Invalidly published in *Cronartium*; *P. stalactiforme* Arth. & Kern.

Diagnostic traits separating these fungi are few, and none is completely satisfactory. One practical and descriptive way to group the pine stem rusts is by the type of disease or symptoms they cause. Four basic effects on hosts have been described (Peterson 1967):

- Stimulation of apical meristems (*C. conigenum, C. strobilinum*--the cone rusts) causes the host to proliferate pithlike tissue and primary vascular tissue (although lateral meristems are stimulated as well).

- Stimulation of lateral meristems (*C. quercuum, P. harknessii*) causes hosts to proliferate secondary tissues through hyperplasia, usually resulting in galls. Western gall rust (*P. harknessii*) also causes perennial cankers by eventually killing tissues from the center of the infection (primary gall) outward. The fungus continues to grow centrifugally, causing a series of secondary galls (ridges) before these, in turn, also become necrotic. Some of these infections remain viable for over two centuries (Peterson 1961).

- Stimulation lacking (*C. occidentale, C. ribicola, C. comandrae, C. appalachianum, P. pini, P. stalactiforme*). This largest group of stem rusts usually causes no direct stimulation of tissues, although hormonal imbalance may induce lateral buds not in direct contact with the pathogen to grow. Infections eventually result in cankers. *P. stalactiforme* does cause minor lateral meristem stimulation (as well as causing limb rust, see below), but its predominant symptom is an elongate canker.

- Systemic infection (*C. arizonicum, P. filamentosum, P. stalactiforme*--the limb rusts). These remarkable rust fungi infect their hosts systemically, proliferating in mature tracheids and penetrating radially into the xylem of large trees (Peterson and Shurtleff 1965). Infection is through a small twig, after which mycelium grows through a branch to the bole, up and down the bole, and back out into new limbs. Eventually infected branches die, giving the tree a window like appearance at mid crown. The mycelial thalli thus formed from single infections may be unique among parasitic fungi in their extension and biomass. Aecia of *C. arizonicum* and *P. filamentosum* have a distinctive long, tonguelike shape. These two species cause limb rust on all hosts they infect, but *P. stalactiforme* causes limb rust only on Jeffrey pine (*Pinus jeffreyi*), and then only from the central Sierra Nevada southward. On other host species and in other places, it causes cankers (Peterson 1968).

Because they do not unambiguously separate the causal fungi, symptoms cannot be the criteria for taxonomic classification. Peterson (1967, 1973) emphasized spore size and morphology, particularly the degree of aeciospore wall modification, but acknowledged their limitation in discriminating some species. Host specificity will sometimes separate morphologically similar species and is the basis for the subspecific designation of *forma specialis* (special form).

Several species with narrow host ranges or distinctive morphology are straightforward enough, such as *C. appalachianum, C. comandrae, C. comptoniae*, and *C. occidentale*. Others form complex taxa that have not been fully worked out. We will focus the remainder of our discussion on these.

C. ribicola *Complex*

Until relatively recently, *C. ribicola*, the white pine blister rust, was thought to be a fairly homogeneous species. Recognition of greater complexity began when young plantations of native Korean pine (*P. koraiensis*) in both Korea and Japan started coming under severe attack from blister rust (Yokota et al. 1975; La and Yi 1976). Eastern white pine (*P. strobus*), planted as an exotic, also became heavily infected in Japan, although still not so badly as Korean pine. This was cause for some alarm because Korean pine had been highly resistant in European and North American trials. Moreover, most of these plantations were associated with infected *Pedicularis* alternate hosts (as opposed to *Ribes*), suggesting that the rust was *C. kamtshaticum*. This rust had long been known on scrophulariaceous hosts (*Pedicularis* and *Castilleja*) from the Kamchatka peninsula down through the Kuril Islands to northern Japan. Its connection to the aecial stage of *Peridermium kurilense* on *Pinus pumila* was presumed but never proven by artificial inoculation (Wicker and Yokota 1976). *C. ribicola* on *Ribes* had also been observed on the island of Hokkaido since the turn of the century. Inoculations conducted in Japan to clarify host relationships showed that aeciospore isolates collected from both *P. pumila* and *P. strobus* were complexly related (Yokota and Uozumi 1976). The isolates fell into one of three types: those that would infect

- both *Ribes* and *Pedicularis*, designated *C. ribicola* f. sp. *pedicularis*--this discovery undermined the basis for a separate species in *C. kamtshaticum*;

- *Ribes* only, designated *C. ribicola* f. sp. *ribicola*; and

- *P. pumila* only--an autoecious form, the first white pine-to-white pine stem rust discovered, subsequently named *Peridermium yamabense* Saho & I. Takahashi (Saho 1981).

No trials with *Castilleja* were reported. These findings prompted new international trials. In Germany, *Ribes*, but not *Pedicularis*, was successfully inoculated with isolates of *P. strobus*, whereas the converse was true in Korea with isolates from *P. koraiensis*, suggesting an additional type that only attacks *Scrophulariaceae* (Stephan and Hyun 1983). Hiratsuka and Maruyama (1976) reported successful inoculation of *Castilleja* with Canadian isolates from *P. monticola* and *P. albicaulis* in a single trial, but Hunt (1984) was unable to infect scrophulariaceous hosts in field tests in British Columbia.

Meanwhile, two additional races of white pine-to-white pine *Peridermia* infecting *P. pumila* on isolated mountain tops in Hokkaido and Honshu were found (Saho 1987). These could be distinguished from each other and the previously described *Peridermium yamabense* by a combination of characteristics, including nuclear number, type of aeciospore wart ornamentation, and germ tube branching. Struck by the similarity of one of these races (Kurikoma) to *Endocronartium* (= *Peridermium*) *pini* Y. Hiratsuka, Saho conjectured that it might be a *forma specialis* of the latter.

Little is known about the race in India on Himalayan blue pine (*P. griffithii*) and *Ribes*, but Peterson (1967) was emphatic on its morphological distinction in the aecial stage from both *Peridermium strobi* (= *C. ribicola*) and *P. kurilense* (= *C. kamtshaticum*, now considered part of *C. ribicola*: Yokota and Uozumi 1976). Much remains to be learned of the genetic amplitude of this complex.

C. flaccidum *Complex*

This complex is the most widespread of all pine stem rusts geographically (if one excludes *C. ribicola* artificially introduced into North America and Europe), ranging throughout Eurasia from Spain and Great Britain to Japan and the Philippines. It has the most alternate hosts, with members in ten different families (Table 3, Fig. 6), and has had more synonyms than any other *Cronartium* or *Peridermium*. This alone suggests its variability, which is extensive, but Peterson (1973) considered all the Eurasian *Cronartia* on *Sylvestres* hosts, including *C. delawayi*, *C. gentianum*, and *C. himalayense* conspecific in *C. flaccidum*. Van der Kamp's (1968) review concluded that the complex comprised at

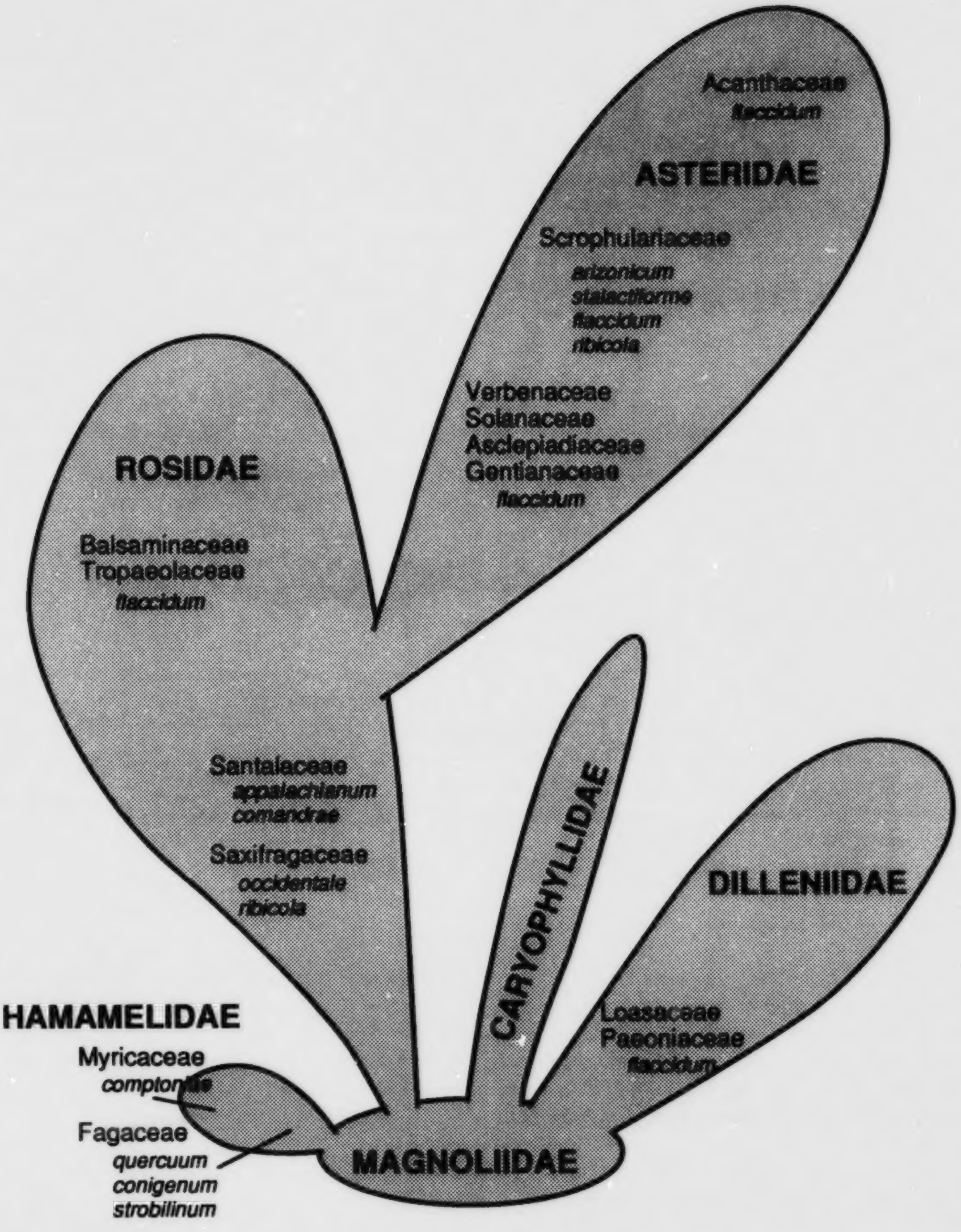
Acanthaceae
flaccidum
ASTERIDAE
Scrophulariaceae
arizonicum
stalactiforme
flaccidum
ribicola
Verbenaceae
Solanaceae
Asclepiadiaceae
Gentianaceae
flaccidum
ROSIDAE
Balsaminaceae
Tropaeolaceae
flaccidum
Santalaceae
appalachianum
comandrae
Saxifragaceae
occidentale
ribicola
CARYOPHYLLIDAE
DILLENIIDAE
Loasaceae
Paeoniaceae
flaccidum
HAMAMELIDAE
Myricaceae
comptoniae
Fagaceae
quercuum
conigenum
strobilinum
MAGNOLIIDAE

Figure 6. Alternate host families of pine stem rusts. Lobes indicate relative size of subclasses of dicots (bold type) in phylogenetic sequence from bottom to top (adapted from Cronquist 1968). Stem rusts in *Cronartium* are in italics.

least one race or special form that alternates to *Gentianum* and *Paeonia* but not other dicots; another that alternates to many dicotyledons (including *Paeonia*) but not *Gentianum*, and an autoecious form (*P. pini*).

Gibbs et al. (1988) described characteristics of this complex in Britain that further confused the picture. They examined nuclear and germ tube behavior of isolates from Scotland and England and were surprised to find that the long recognized autoecious *P. pini* in Scotland had binucleate, long, nonseptate germ tubes typical of heteroecious *Cronartia* (including *C. flaccidum*). Furthermore, the "Thetford" form from East Anglia had short, uninucleate, septate germ tubes typical of autoecious *Peridermia* (or *Endocronartium*, including *E. pini*: Hiratsuka 1968, 1969; Hiratsuka et al. 1966), yet some isolates had the ability to infest *Paeonia*! Other isolates apparently could infect pine directly. They concluded that there must be at least two races of *P. pini*, the Scottish long-tube form and Hiratsuka's (1968) short-tube form. They also raised again the issue of facultative heteroecism--the possibility that the same spore form of a rust can infect both primary and alternate hosts. This was first proposed by Meineke (1929) but never satisfactorily proved (Wagener 1964).

C. quercuum *Complex*

The gall-forming pine-oak rusts were formerly assigned to two species, *C. quercuum* of eastern North America and Asia (the only rust common to North America and Asia) and *C. fusiforme* of southern USA. There were few or no morphometric differences or an alternate host specificity to distinguish the two; distinction was based on gall shape (globose or fusoid) and unclear host specificities among southern and eastern species of pines in subsections *Australes* and *Contortae*.

Burdsall and Snow (1977) partially resolved the problem by relegating *C. fusiforme* to synonymy with *C. quercuum* and erecting four special forms (f. sp. *banksianae*, *echinatae*, *fusiforme*, and *virginianae*) based on data of host specificities. However, they did not deal with the form parasitizing Asiatic pines in *Sylvestres*. Peterson (1967) found no significant morphological differences between North American and Asian isolates, but Powers et al. (1988) reported many physiological, phenological, and morphological differences (see also Powers et al., 1990). The cone rusts (*C. conigenum* and *C. strobilinum*) are related to *C. quercuum* in having the same alternate hosts in *Quercus*; together, they are often known as the pine-oak rusts.

C. coleosporioides *Complex*

This diverse group of rusts on western North American hard pines causes very different diseases but is united in having common alternate hosts in *Scrophulariaceae* (or lacking alternate hosts, i.e., are autoecious). The group includes several limb rusts, a canker rust, and a gall rust. Inoculations of alternate hosts with aeciospores from some of the species produce morphologically distinct urediospores (Peterson 1973). Their inclusion in *C. coleosporioides* is thus a gross oversimplification. The limb rusts form a subcomplex on their own, which formerly were all described as three races of *P. filamentosum*:

- a heteroecious race (Coronado, named for the national forest where it was discovered) was recently described as *C. arizonicum* (Cummins 1984), and

- two autoecious races (Powell and Inyo), both with short septate germ tubes typical of autoecious species, can be distinguished from each other by morphological and phenological traits (Peterson 1968).

P. stalactiforme also causes a limb rust but only on Jeffrey pine and only in certain geographic areas. On lodgepole (*P. contorta*) and other pine hosts, it causes bark swelling and very elongate cankers. *P. stalactiforme* alternates to hosts in *Scrophulariaceae* and is clearly a distinct species but is not validly described in *Cronartium*.

P. harknessii, the western gall rust, is usually considered autoecious. Although several reports of alternation to *Scrophulariaceae* exist, most attempts to verify these claims experimentally have failed (Peterson 1967, 1973). This rust is highly variable, with one of the widest host ranges--four subsections of pine, (compared with only one for the *filamentosum* subcomplex--and one of the widest geographic distributions: from the Pacific to the Atlantic coasts and from sea level to over 3500 m elevation (Peterson 1960). An albino race is widespread in western North America on ponderosa pine (*Pinus ponderosa*) (Mielke and Peterson 1967). Symptomologically, *P. harknessii* has closer affinities with *C. quercuum* than its cohorts in *C. coleosporioides*.

Clearly, much remains to be done to resolve some of these taxonomic relationships, but new approaches are needed. Isozyme analysis is a promising technique that has long been available but only recently exploited (Vogler et al. 1988; Tuskan and Walla 1989; Powers et al. 1989). Starch gel electrophoresis is efficient because 20 or more marker loci can be analyzed simultaneously on many different isolates. Results in our laboratory have so far indicated a remarkable uniformity of structural genes within populations of the wide-ranging western gall rust (*P. harknessii*), making comparisons among populations unambiguous (Vogler et al. 1988). Initial comparisons of the few species we have examined show the same pattern of homogeneity within taxa and heterogeneity among taxa as in western gall rust (unpublished data). Similarly, Powers et al. (1989) obtained clear separation of five species as well as the four *formae speciales* of *C. quercuum*, using polyacrylamide gel electrophoresis. Tuskan and Walla (1989) found more variability than did Vogler et al. (1988) or Powers et al. (1989) within populations of *P. harknessii* and *C. quercuum* f. sp. *banksianae* in North Dakota and Minnesota but were able to separate the two species. These and other molecular techniques are promising and need to be pursued systematically for the genus.

Phylogeny and Evolution of Stem Rusts

Rusts are generally thought to be an ancient lineage whose original hosts were primitive plants, such as ferns and mosses, possibly arising in the warm, moist forests of the Carboniferous Period. At this time they were probably autoecious, with a relatively simple life cycle and a spore stage homologous with teliospores. A unique adaptation acquired early in rust evolution was heteroecism--the ability simultaneously to parasitize their original host as well as alternate, or secondary, hosts in completely different families of evolutionarily more advanced taxa. This intriguing paradox--of extreme specialization on the one hand, often requiring a matching gene-for-gene fit to hosts, coupled with such apparent promiscuity on the other--has been difficult to rationalize. Leppik (1967) coined the terms biological specialization and biogenic radiation for these opposing trends. Nevertheless, such radiation is usually not indiscriminate and is often confined to a single family or even species. *Cronartium flaccidum*, with its profusion of alternate hosts in ten dicot families, is a striking exception. Perhaps heteroecism, accompanied by acquisition of new spore forms adapted to seasonal climates, occurred in response to some drastic environmental change such as the colder, drier climate of the Permian Period (which also gave rise to the conifers). Together, these adaptations increased both the genetic and ecological amplitude of the lineage that became the aecial rusts, while retaining for them a secure base on their primary hosts until well established on secondary hosts.

The first major new host group was the conifers; extant fern-conifer pathogens are *Hyalospora*, *Milesina*, and *Uredinopsis*. From the conifers, rusts radiated successively to primitive, then more advanced dicots (meanwhile abandoning ferns, then conifers, as primary hosts), culminating with the dicot-monocot relationship where the overwhelming majority of species exist today. The process of radiation, establishment, and reradiation Leppik (1953) called "climbing the hologenetic ladder" of ecologically more opportunistic host species. Autoecious and microcyclic rusts are usually considered to be derived from heteroecious and macrocyclic parent species.

The theory of coevolution of rusts with their hosts is classic (Leppik 1953, 1967; Savile 1971; Hiratsuka and Sato 1982). It implies that the phylogenetic history of one member of the pair ought to reflect that of the other, although it has more often been used to deduce rust phylogenies from hosts than vice versa. It has considerable intuitive appeal and heuristic value but is not universally accepted; Hart (1988) recently concluded that host transfer was at least as frequent as cospeciation (coevolution) of rusts with hosts, based on cladistic analysis of 30 rust genera. The two concepts are not mutually exclusive, and we accept the classic view in our consideration of pine stem rust evolution. It is much easier to demonstrate at higher taxonomic ranks (classes, orders), but we assume that the same principles apply at lower levels as well--that is, that phylogenetically more primitive taxa of hosts harbor the more primitive rusts. Lack of a fossil record in *Cronartium--Peridermium* (as with most fungi) presently restricts our inferences to phylogenetic evidence in any case.

This evidence is recapitulated in Figures 6 and 7 for dicot and pine hosts respectively. Dicot hosts of *Cronartium* are in four of six of the subclasses. Figure 6 shows subclass and family relationships in phylogenetic sequence (following Cronquist 1968) from primitive (bottom) to advanced (top). There are no hosts in the most primitive subclass (*Magnoliidae*) but there are in three of four directly derived lineages. Although most species are limited to a single host family (sometimes a single genus), there are groups of rusts that share common families in *Fagaceae* (3 species), *Saxifragaceae* (2), *Santalaceae* (2), and *Scrophulariaceae* (4). *C. comptoniae* stands alone in *Myricaceae*, but *C. flaccidum* is nearly ubiquitous, occurring in ten families (of 14 total) and in nine of these exclusively. These range from the most primitive (*Paeoniaceae*) to most advanced (*Scrophulariaceae*, *Acanthaceae*). At least two races, or *formae speciales*, are known on *Asteridae* hosts (see previous section), and perhaps more await discovery in the vast geographic and host range of this species complex. *C. ribicola* also has races or *formae speciales* in both relatively primitive (*Saxifragaceae*) and advanced (*Scrophulariaceae*) families.

The rusts in the *Fagaceae* group are undoubtedly related to each other, as are those in the *Saxifragaceae* and *Santalaceae* groups, by symptomology as well as primary hosts. A distinct exception is the very disparate group of rusts sharing scrophulariaceous hosts. These include limb and canker rusts on subgenus *Pinus* in western North America and canker rusts on subgenus *Strobus* in Asia and appear to represent a case of convergent evolution.

The evolutionary sequence of the stem rusts is shown against the phylogenetic background of their primary pine hosts in Figure 7. Correlated evolution again is assumed, and speciation events of rusts are approximately superimposed onto differentiation of host groups. We recognize that the scheme is oversimplified and often conjectural, with timing of speciation events having only relative value. Nevertheless, the evidence from primary and alternate hosts is reasonably consistent.

We must assume that the primordial *Cronartium* evolved before the major split of the main subgenera of pines (*Pinus* and *Strobus*), undoubtedly after the breakup of Pangaea into Laurasia and Gondwanaland but before the Laurasian split. This early progenitor followed subgenus *Strobus* as the ancestral *C. ribicola* and subgenus *Pinus* as the ancestral *C. flaccidum*. Both of these lineages persist, but

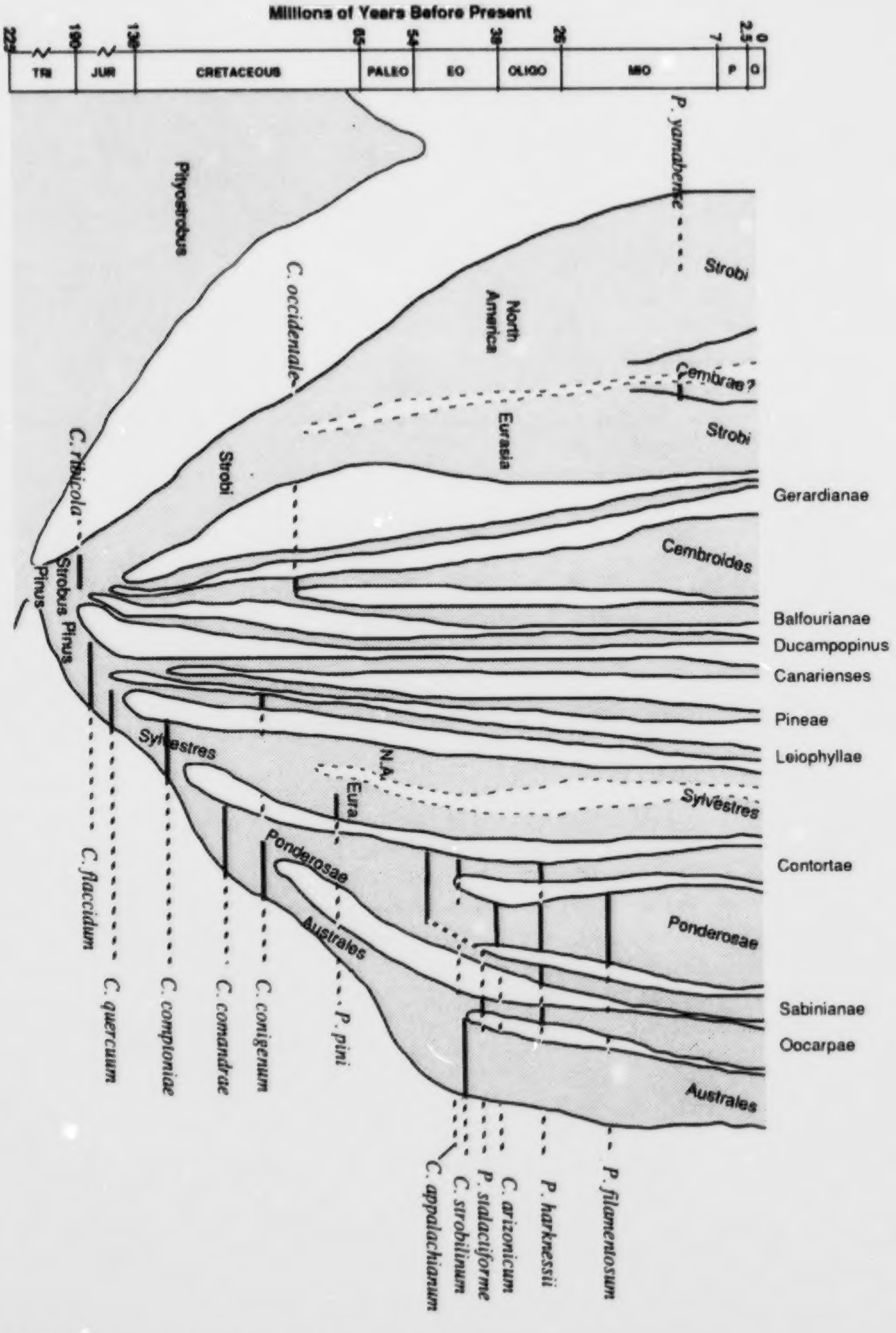
Millions of Years Before Present
225
190
136
65
54
38
26
7
2.5
0
TRI
JUR
CRETACEOUS
PALEO
EO
OLIGO
MIO
P
Q
Pityostrobus
P. yamabense
Strobi
North America
Cembrae?
Eurasia
Strobi
C. occidentale
Strobi
C. ribicola
Strobus
Pinus
Pinus
Gerardianae
Cembroides
Balfourianae
Ducampopinus
Canarienses
Pineae
Leiophyllae
Sylvestres
N.A.
Eura.
Sylvestres
Contortae
Ponderosae
Ponderosae
Australes
Sabinianae
Oocarpae
Australes
C. flaccidum
C. quercuum
C. comptoniae
C. comandrae
C. conigenum
P. pini
C. appalachianum
C. strobilinum
P. stalactiforme
C. arizonicum
P. harknessii
P. filamentosum

Figure 7. **Hypothesized phylogeny of stem rusts in *Cronartium--Peridermium*** (italics) **superposed on phylogeny of pine hosts** (cf. Fig. 2). Horizontal bars indicate relative order of differentiation of lineages leading to modern stem rust species, as inferred from present host-rust affinities and host phylogenetic histories.

all the other taxa split off along the way. Evidence supporting this is the relative antiquity of alternate hosts in *Ribes* and *Paeonia*, as well as the two primary hosts in *Strobi* and *Sylvestres*.

After the Laurasian split, the white pine rusts differentiated, along with their hosts, into *C. ribicola* on subsections *Strobi* and *Ribes* in Asia and *C. occidentale* on subsection *Cembroides* and *Ribes* in North America. Later in Asia, *C. ribicola* radiated to hosts in *Scrophulariaceae* as well, then later still split off an autoecious variant on *P. pumila*, if our chronology of the segregation of subsection *Cembrae* is correct (see Fig. 7).

Phylogeny of the hard pine (subgenus *Pinus*) stem rusts is more complex. Although *C. flaccidum* is ancient by the criteria of the antiquity of its primary host in *Sylvestres* (also *Canarienses* and *Pineae*) as well as one of its alternate hosts (*Paeonia*), it is anomalous in the diversity of alternate hosts it attacks and the relative phylogenetic youth of most of them. We can rationalize this if we assume that much of this radiation occurred later--for example, to the *Scrophulariaceae*. Why *C. flaccidum* did not follow North American members of *Australes* after the Laurasian split is unclear. *Peridermium pini* may have derived from *C. flaccidum* at some point after this time.

Pine-oak rusts were another early lineage preceding the Laurasian split. *C. quercuum* causes galls on species in subsection *Sylvestres* in Asia and *Australes* and *Contortae* in North America. Although nominally the same species at present, it seems unlikely that the stem rusts on these two continents will remain so because of recently described differences discussed earlier. Later offshoots from the ancestral *C. quercuum* were the cone rusts *C. conigenum* on western North American *Ponderosae* and *Leiophyllae* and eastern *Australes*, and *C. strobilinum* on *Australes* only. *C. comandrae*, with alternate hosts in *Santalaceae*, has the widest primary host range of pine stem rusts endemic to North America (Table 3), but a putatively derived species (*C. appalachianum*) is limited to a single pine species of both pine (*P. virginiana*) and alternate host (*Buckleya distichophylla* (Nutt.) Torr.).

Of the western group of North American rusts united by scrophulariaceous alternate hosts, *Peridermium stalactiforme* (a *Cronartium* without formal or valid description) might be ancestral to *C. arizonicum* because of its wider host range in *Contortae*, *Ponderosae*, and *Oocarpae*, compared with *Ponderosae* alone for *C. arizonicum*. The autoecious *P. filamentosum* complex probably derived from *C. arizonicum*. *P. harknessii*, the western gall rust, is problematical. It has the widest host range of any *Peridermium* (four western North American subsections), and it causes symptoms unlike *C. arizonicum* or any of the other limb rusts, even though it has been grouped formally in the *C. coleosporioides* complex because of dubious claims that it can occasionally infect species of *Scrophulariaceae*.

Centers of Diversity

Earlier we described how centers of genetic diversity can develop where the environment is variable enough over both space and time to reorganize (without exterminating) available genetic variability through natural selection. For pines, such secondary centers of speciation and diversity began to develop during the Eocene and continued through the Pleistocene in regions like the North American southwest (especially Mexico) and Japan, where the predominantly north-south topographic grain allowed plant populations to migrate with the ebb and flow of glaciers. These two situations contrast with northern Europe, where east-west mountain barriers restricted migration during glacial advances and led to reduced species diversity.

We may assume that a similar dynamic applies to the pine stem rusts and their alternate hosts as well. The pattern we see is different parts of the pathosystem alternately expanding and contracting along environmental gradients in response to major climatic fluctuations over time. During this process, populations alternately become fragmented and continue their short-term evolution in isolation, only to reunite later, forming new combinations of variability. Since the last glacial ended so recently, it is not surprising that many anomalous and complex patterns of variation exist in extant populations.

Japan is an unusual center of diversity for many conifer rusts as well as their hosts. In Table 4, different pines, stem rusts, and alternate hosts are shown overlapping in elevation from near sea level to timberline. *C. quercuum* and *C. flaccidum* share a common pine host (*P. densiflora*), and *C. flaccidum* and *C. ribicola* share a common alternate host (*Pedicularis*). Approaching timberline, *C. ribicola* overlaps both *Pinus koraiensis* and *P. pumila* and also its presumed autoecious derivative, *Peridermium yamabense* on *P. pumila*. The existence of at least three distinct morphological and physiological races of autoecious white pine rusts on different mountaintops of both Honshu and Hokkaido (Saho 1987) is interesting. It suggests that perhaps the host populations never descended low enough in elevation during the full glacial to make contact with each other, remaining isolated as montane islands for longer than one glacial cycle, or that these rusts are capable of more rapid evolution (in any one glacial cycle) than might have been expected.

Are any of these rust populations exchanging genes? This question is critical to defining the genetic amplitude of these species and even to our concept of the species. It leads to broader questions that address taxonomic and evolutionary problems remaining for the pine stem rusts and that have important implications for domestication strategies for pine crops in jeopardy from these pathogens:

Table 4. Primary and alternate hosts of pine stem rusts (*Cronartium--Peridermium*) in the central mountain region of Honshu, Japan (adapted from Hama 1987 and Kakishima et al. 1984)

Primary host	Elev. (m)	Stem rust	Alternate hosts
P. thunbergiana	400-600	*C. quercuum*	*Quercus serrata. Q. dentata*
P. parviflora	500-900	None	
P. densiflora	500-1200	*C. flaccidum*	*Paeonia albiflora* f. *hortensis*, *P. obovata*, *P. suffruticosa* *Pedicularis resupinata* var. *caespitosa*
		C. quercuum	*Quercus serrata*, *Q. dentata*
P. koraiensis	1200-2600	*C. ribicola*	*Ribes sinanense* *Pedicularis euphrasioides*, *P. chamissonis* var. *japponica*, *P. resupinata*, *P. yezoensis*
P. pumila	2300-2900	*C. ribicola*	*Ribes sinanense* *Pedicularis euphrasioides*, *P. chamissonis* var. *japponica*, *P. resupinata*, *P. yezoensis*
		P. yamabense	None

- What is the genetic structure of stem rust species? That is, what is the magnitude of their genetic variation, how is it distributed geographically within and among populations (or among hosts), and how is it maintained?

- Is the sexual cycle really functional in heteroecious species? If so, is sexual behavior predominantly outbreeding or inbreeding?

- What are the genetic implications of autoecism, or endo-type sexual cycles? Does gene exchange occur among individuals and populations of these species, or have they arrived at evolutionary dead ends?

These questions are rooted in the phylogenetic history of these rusts and their hosts and can best be explored in centers of gene diversity such as Japan and western North America. Increased use of existing and emerging techniques of molecular biology will hasten their solution.

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HISTORY AND MAJOR ACCOMPLISHMENTS OF FUSIFORM RUST RESEARCH ON SOUTHERN PINES

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Tremendous progress has been made in fusiform rust research during the past 25 years. To maintain a proper perspective, however, it is necessary to go further back in time. Important findings during the first half of the century are sometimes overlooked, but they formed the basis for recent progress. This disease, caused by the fungus now called *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*, was first described in 1896 on loblolly pine in Alabama. The pathogen is native and it attacks native hosts. This macrocyclic, heteroecious rust, which alternates between hard pines and oaks, is increasing in severity as forest management practices become more intensive.

In a *Journal of Forestry* article entitled "Geographic Source of Loblolly Pine Seed", Phillip Wakeley (1944) revealed some key facts about the fusiform rust situation on loblolly pine. Findings were based on plantings made as early as 1926 in Louisiana of loblolly seedlings derived from Texas, Arkansas, Louisiana, and Georgia seed sources. At age 15, rust infection on the Georgia trees was 80%. In contrast, the Livingston Parish, Louisiana, and the Texas trees had 3% and 32% infection, respectively, and the Arkansas trees had 52%. The variation in resistance to fusiform rust among these geographic seed sources of loblolly pine surprised early researchers. The study was later enlarged by Wakeley and Wells (1966), and additional geographic sources were added. These results show us that fusiform rust was not rare, uncommon, or insignificant in the 1930s. Obviously, when conditions were favorable, rust could be a problem even then.

Another important paper published in the 1940s was Hedgcock and Siggers' (1949) "A Comparison of the Pine-Oak Rusts". The paper described work from 1908 through the early 1940s. It reviewed a range of topics including distribution and host range of pines and oaks, and inoculation studies with the various isolates or forms of what they called at the time *Cronartium cerebrum* Hedge. & Long., the eastern gall rust, compared to fusiform rust and cone rust. The large number of times that this paper has been cited in later publications gives a good indication of how important this research was, and what a treasured source of information it has become. This paper was written primarily by the junior author Siggers after Hedgcock's death. Siggers himself did a great deal of work on epidemiology and control of fusiform rust. His papers on weather and outbreaks of rust of southern pines and on various control problems are often quoted today (Siggers 1949).

Beginning around 1950, people began to think seriously about tree improvement work with southern pines. Barber et al. (1957) wrote a landmark publication on disease resistance and variation within southern pines in response to infection by fusiform rust. They demonstrated significant differences in response to infection by fusiform rust on open-pollinated progeny from a group of slash pines. At about the same time, Fred Jewell, Birch Henry, and several others were finding excellent resistance to rust in short leaf hybrids with slash and loblolly pine (Henry and Bercaw 1956). All of this work provided a firm foundation for tree improvement programs in the South, which now involve several universities, private industries, and federal and state agencies. As a result, the southern United States leads the world in forest tree improvement technology, and is producing and planting over a billion tree seedlings every year.

To evaluate the results of early tree improvement efforts, Fred Jewell (1960) developed the first effective procedure for inoculating seedlings with the fusiform rust fungus. He found he could infect pine seedlings successfully in a moist-tent inoculation chamber.

Another area of major effort in fusiform rust research was histology. Jackson (1958), at the University of Georgia, and Fred Jewell et al. (1962) at the Gulfport, Mississippi, laboratory were leaders in this work. Their effort and later work by Miller et al. (1976) and Charlie Walkinshaw (1978) resulted in a good knowledge of the microscopic detail involving infection and colonization of the pines by the fusiform rust organism.

During the late 1950s and early 1960s, quite a bit of work was done on the control of fusiform rust disease in forest tree nurseries. Infection in forest tree nurseries often was extremely high. To give you an idea of how high, the State Forester of South Carolina lost his job because of rust losses in that State's nurseries. Up to 85% of the nursery seedlings were infected. At the time, several people, including Siggers (1955) and Foster (1956), were working on spray programs that would control the rust in these nurseries. The fungicide spray schedules they developed became standard treatments for almost 30 years, until the introduction of the systemic fungicide Bayleton a few years ago.

In the late 1960s, some excellent work was done on effects of environmental factors on fusiform rust infection. Glenn Snow published a series of papers about dispersal of basidiospores, weather conditions affecting infection, and the time required for the infection after exposure to the inoculum (Snow 1968a,b; Snow and Froelich 1968; Snow et al. 1968). During that period, work also was being carried out on site-hazard evaluation. Findings about relative amounts of the oak alternate hosts, soil types, and some other factors, helped to delineate areas where rust will be severe (Schmidt et al. 1972; Schmidt et al. 1974).

In 1971, Felix Czabator published a very comprehensive literature review on fusiform rust. His review is certainly one of the best available and is an excellent starting point for understanding this topic.

In the late 1960s and early 1970s, tremendous progress was made in understanding fusiform rust. During these years, several new and promising inoculation procedures were developed and evaluated. Snow and Kias (1972) developed a procedure in which individual seedlings were inoculated with a few basidiospores at a specific spot on the stems. The concentrated basidiospore spray (CBS) system was developed by a team of scientists at Athens, Georgia (Matthews and Rowan 1972). The CBS system is advantageous because each seedling receives a standardized inoculum concentration, and large numbers of seedlings can be inoculated under uniform conditions. The Forest Pest Management group of the USDA Forest Service at Bent Creek, North Carolina, now uses the CBS system to screen seedlots on request for all tree improvement groups (Anderson and Powers 1985).

Also in the early 1970s, a large-scale fusiform rust incidence survey was carried out in the southern United States (Phelps 1973). This effort was the largest of its kind ever carried out. The publication is still the primary source of information on the distribution of rust throughout the South, and certainly is one of the most frequently cited works on fusiform rust. In the mid-to-late 1970s, research was done on the economic impact of rust (Powers et al. 1975), and estimates of eventual rust-associated mortality related to incidence levels during the early years of a pine plantation (Sluder 1977). This work made it possible to make good estimates of actual dollar losses.

Research on breeding and selecting for resistance also progressed rapidly in the early 1970s. Several tree improvement cooperatives in the South set up what they referred to as "specialty" orchards, containing clones of known resistance to fusiform rust (Zobel et al. 1971). The Southeastern Forest Experiment Station and the Georgia Forestry Commission developed a rust-resistance orchard that produced some 5 million resistant seedlings in 1988 (Powers et al. 1976). Meanwhile, a University of Florida group demonstrated that levels of rust resistance could be improved by roguing heavily infected stands (Goddard et al. 1975).

In the mid-1970s, mycological work yielded a new designation of the *Cronartium quercuum* group by "formae speciales" (Burdsall and Snow 1977). Although it lengthened the name that we have to use for fusiform rust from *Cronartium fusiforme* to *Cronartium quercuum* f. sp. *fusiforme*, I think it put us on a very logical and sound basis, since morphologically identical organisms are separated on the basis of host preference.

Also in the mid-1970s, some extensive research was performed on pathogenic variation in the fusiform rust population across the South. Glenn Snow carried out research on slash pine (Snow et al. 1975), while we at Athens took a comparable look at pathogenic variation of fusiform rust over the range of loblolly pine (Powers et al. 1977). This research demonstrated that the organism was extremely variable, and that there could be as much variation within a single pine plantation as there was from county to county, or even from state to state.

Another tremendous step forward was use of the systemic fungicide Bayleton in nurseries (Rowan 1980; Snow et al. 1979). This material gives excellent control of the fusiform rust infection with only three to four sprays per season as compared to the 30 to 40 sprays with Ferbam. Bayleton now is routinely used for control of rust in nurseries throughout the South.

Research is now under way on *in vitro* culture of the organism and testing for resistance by using single-spore cultures on clonal lines of the pine host developed by micropropagation. Use of specific isolates of the rust fungus will lead to greater understanding of the mechanisms of resistance and of resistance factors in the host.

Table 1 shows what I consider to be the greatest achievements in fusiform rust research from 1908 to 1980. I have excluded work begun in the 1980s, since much of it is still incomplete. The achievements are listed somewhat chronologically, and I am sure that others who have worked on fusiform rust would come up with their own list.

Some important obstacles are impeding our progress in understanding fusiform rust. At the head of the list is the fact that the life cycle of the fusiform rust fungus has its repeating or uredinal stage on the oak alternate host. In cereal rust, the repeating stage develops on the commercially important crop, exposing that crop to clonal lines of the pathogen, or pathogenic races. However, with fusiform rust, nuclear fusion and reduction division occur prior to the production of the basidiospores that infect the pines. As a result, pines are exposed to a highly variable population of the pathogen--so variable that some percentage of the seedlings from a tree are almost always infected by a certain proportion of the basidiospores.

Table 1. The 10 most important advances in fusiform rust research 1908-80

1. Geographic areas of loblolly resistance
2. Variation in rust susceptibility in slash pine
3. Ferbam for forest tree nursery sprays
4. Development of rust resistance orchards
5. The fusiform rust incidence survey
6. Development of CBS inoculation systems
7. Economic analysis of financial losses of rust
8. Research of pathogenic variation of *Cronartium quercuum fusiforme*
9. Research on site hazard evaluation
10. Bayleton sprays for nursery controls

Another problem is that we know very little about the genetics of resistance in pines or oaks. To study resistance mechanisms, we need to limit heterogeneity in the pine host and in the pathogen population. Until both are standardized, we can only generalize about the genetics of this system. Ultimately, the chromosome maps being developed for loblolly pine should give us the ability to fine-tune our efforts.

Another difficulty in working with pines as compared to cereals is that we are dealing with a normally outcrossing host species as compared to the highly inbred, self-pollinated wheat. Loblolly pine, for example, has natural hybrids with almost all of the other species of southern pines. All of this complexity severely limited our understanding of the genetic basis of resistance.

Existing inoculation systems provide reliable estimates of relative susceptibility or resistance in 6 to 9 months. Speeding that process would be valuable. By analyzing isozymes of pollen, we have quickly and fairly reliably identified resistant loblolly pines (Powers, Lin, and Hubbes 1986), but not slash pine. Some enzyme systems not yet tested may be indicators of resistance in slash pines. Some other new technological approaches such as restriction fragment length polymorphisms (RFLP) might be used to speed things along. With our improved technology, we are now able to grow the rust fungus in culture, but growth is agonizingly slow. Certainly, improvement here would be valuable. In addition, we need better techniques to inoculate pines with hyphal cultures of the organism.

Another difficult area is the lack of an accurate means of predicting site hazard on a localized basis. We have methods for estimating rust hazard over broad areas, but on a specific site the best approach often is to simply go and look at adjacent young stands. A means of accurately measuring or pinpointing hazard on a very localized level would be of immense value to forest land managers.

While some difficulties are impeding progress, tremendous gains have been made. Those of us that were working in this area in the 1950s and early 1960s are particularly aware of the advances that have been made--of how far we have come over the last 25 years.

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HISTORY AND ACCOMPLISHMENTS OF WHITE PINE BLISTER RUST RESEARCH IN THE USDA FOREST SERVICE

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The blister rust fungus, *Cronartium ribicola* J.C. Fisch., was introduced into western North America at Point Grey near Vancouver, B.C., Canada, in 1910 (Lachmund 1926). This full-cycle stem rust of five-needle pines came to western North America in the late 1800s in a shipment of eastern white pine (*Pinus strobus* L.) originating in France (Mielke 1943). It spread into the interior of British Columbia by fall 1917 (Lachmund 1926). Cankers found in 1927 in northern Idaho were dated back to 1923 infections (Mielke 1943). Thus, in only 13 years, the rust had become established over a major portion of the range of western white pine (*P. monticola* Dougl.) (Hoff 1988).

Blister rust posed major forest management problems in eastern and western forests of two countries, and scientists responded with studies of its nature. Consequently, by 1950, most elements of the basic biology and ecology of the fungus were known (Bingham 1983; McDonald et al. 1981). It was also evident that the principal control measure applied to that time, eradication of the alternate host, *Ribes* spp., was not working in most localities in the western United States (Bingham 1983). Thus, by 1950, the time was ripe for establishment of a research and development program aimed at breeding resistance to blister rust in western white pine.

Such a program was conceived, begun, and directed by R.T. Bingham for the Forest Service, U.S. Department of Agriculture. The more serious scientific aspects of the history of this effort have already been thoroughly discussed (Bingham 1983; Hoff 1988). Our charge for this paper was to be critical of the breeding and research program that evolved under Dick Bingham's leadership. We believe that interesting, and as yet untold, aspects of the white pine blister rust story have their foundation in Bingham's personal qualities and his approach to the conduct of research. To focus on these more human aspects of the history, we define and discuss four Bingham legacies.

HAPPENSTANCE PLAYED A ROLE

This story begins in 1936 when Bingham journeyed from northern New Jersey to enroll in the forestry curriculum at the University of Idaho. Even at this early point, happenstance played a significant role in the ultimate outcome of the blister rust resistance program. Bingham's application to attend the New York State University College of Forestry was rejected 2 weeks before the start of fall term in 1936. He then quickly applied to all other U.S. forestry schools, and the University of Idaho was the first to reply with an acceptance.

Summer employment in a joint University of Idaho-Forest Service research project stimulated his interest in white pine blister rust. After graduating in 1940, Bingham enrolled in graduate school at the University of Idaho to study forest pathology under Professor John Ehrlich. His thesis topic dealt with the biological control of blister rust (Bingham and Ehrlich 1943a, 1943b). A June 1942 graduation with a Master of Science degree was followed by 3 years of service in the U.S. Marine Corps during World War II.

Bingham's discharge was followed immediately by an appointment as a pathologist in charge of rust surveys in the white pine region of northern Idaho and eastern Washington, working out of Spokane, Washington. Further responsibilities included field study of forest soils and rust infection on *Ribes* plants as well as laboratory studies on white pine seeds.

Bingham was in a position to apply his unique understanding of all aspects of the rust problem and concluded that resistance to the fungus was evident in the heavily impacted western white pine stands of northern Idaho as rust-free trees among neighbours supporting hundreds to thousands of cankers. He could arrive at this conclusion because of the unusually large concentration of nearly pure white pine stands in the region and the fact that he had personally climbed and examined large numbers of white pines during the summers of 1946 through 1948 (Bingham 1983).

By the summer of 1949, Bingham was "bootlegging" attempts at controlled pollination and rooting of cuttings from 14 resistant trees in a fashion patterned after the pioneering efforts of Riker and Kouba (1940). Bingham has since become famous for this attempt to pollinate year-old female cones (Fig. 1). Attempts at rooting cuttings were conducted in full view of all visitors to his laboratory in a small window box greenhouse (Bingham 1983). Both efforts were biological failures, but the visible bootlegging spawned the entire program in a rather uncommon fashion. Or was it the common beginning of innovative research programs?

APPROVAL APPEARS

Dick relates the following story in his recent historical account of the program (Bingham 1983):

> The annual, summer 1949 field trip of the Idaho State Land Board (the agency that administers the education-supporting funds coming from timber cut on State lands) was under way, very grandly transported by river-drive, wanigan-raft, down the very remote and beautiful North Fork of the Clearwater River in northern Idaho. One main purpose of this particular trip was for Land Board members and their blister rust administrator guests to consider the acceleration of timber harvesting plans in the State-owned, heavily rusted mature *P. monticola* stands that bordered the river. One exchange between a Land Board member and a now-deceased but then leading blister rust control administrator was leaked to the author about as follows: Land Board member: "I understand that University of Wisconsin researchers are already at work exploring blister rust resistance in *P. strobus*; are your people planning anything along these lines in *P. monticola*?" My informant tells me there was a pregnant pause, and then, as if suddenly remembering the lonely box of cuttings protruding from the 8th floor window in Spokane, or the single, controlled pollination attempt, the blister rust control administrator finally answered, "By golly, we're already working on that!" Perhaps this hearsay deserves some credence, for I can testify that the administrator did, a few weeks later, visit the 8th floor lab and did, as usual, casually glance at the windowbox cuttings, and then did ask me

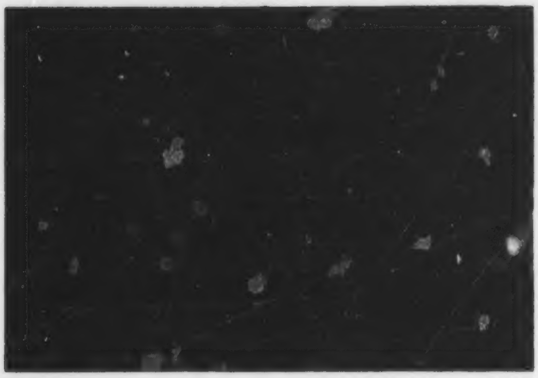

Figure 1. Year-old cones ("Bingham buds") of *Pinus monticola* (U.S. Forest Service photo).

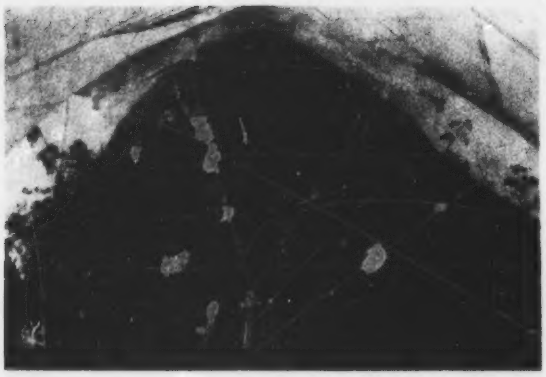

Figure 2. View inside an early large-scale inoculation tent of blister rust infected *Ribes*, white pine seedlings in seedbeds, and misting equipment (U.S. Forest Service photo).

Figure 3. Slow-growing, 20-year-old *Cronartium ribicola* canker on a western white pine inoculated at age two and outplanted to a forest site at age four (U.S. Forest Service photo).

Figure 4. View of the Priest River western white pine vigor-quality plantation at about 25 years after establishment (U.S. Forest Service photo).

pointblank, "Shouldn't we be doing something more toward development of blister rust resistance in *P. monticola*?"

The program got its start because a young scientist was not shy about exhibiting extracurricular attempts at an innovative effort and a research administrator was quick to seize upon an accidental opportunity. At this point, the birth of the program hung by the thinnest of threads. In fact, the dawn of this approach to blister rust control is mostly attributable to a facet of Bingham's personality that his major professor John Ehrlich saw as a weak point. Professor Ehrlich was Bingham's summer supervisor in 1940 and wrote "Somewhat too sure of himself. Occasionally sacrifices thoroughness for speed."

The preliminary bootlegged research and Bingham's ability to work at a fleet pace paid off generously. By the beginning of the pollination season in 1950, a complete program was in place that included a study plan, three scientists trained in pollination techniques for pines, a supporting technician crew to search for additional phenotypically resistant pines, and facilities and equipment to conduct the necessary pollen extraction and record handling (Bingham 1983).

EARLY OBJECTIVES AND WORK

The original proposed study plan contained five major objectives: 1) select, describe, and permanently mark phenotypically resistant trees; 2) vegetatively propagate these selections for protection, increasing, and accumulation; 3) place these collections in areas of heavy rust to effect further testing; 4) test all selected clones and their progeny under uniform and heavy natural inoculation, and convert the clonal test plantations to seed orchards rogued according to progeny test results[1].

Included in the study plan developed in 1949-50 was provision for the first and probably most important legacy: the outplanting of phenotypic selections and progenies of all sorts. Before we discuss this legacy, we need to clear up a loose end. Note that the idea of artificial inoculation was not included in the original five points. The first mention of artificial inoculation was in the 1952 annual report where it was stated that initial inoculation trials were conducted in September and October by J. Kimmey[2]. The first large-scale inoculation was conducted from September 21 to October 2, 1953 on progenies obtained from the controlled pollination of 1950 (Fig. 2)[3].

These progeny were outplanted in 1954[4] and some are still surviving today at three test locations. A very important aspect of this legacy is that no progenies generated by the artificial crossing were discarded outright. All materials were planted at an appropriate forested site, and materials deemed unsuitable for immediate objectives were left to nature's devices. As a result, we have today a stock of materials of known pedigree that have exhibited an extraordinary ability to survive. Today "Bingham's garbage" is a rich source of tolerance and other slow-rusting kinds of resistance mechanisms (Fig. 3). Other outplantings that were part of the initial planning in cooperation with J. Duffield and A.E. Squillace were three vigor-quality plantations. These plantings (Fig. 4) continue to yield valuable information on resistance and growth traits (Steinhoff 1971; Goddard et al. 1985; McDonald et al. 1990).

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[1] Bingham, R.T. 1950. Development of rust resistant white pine. Pages 123-128 *in* Blister rust control work in the northwestern region Jan. 1 to Dec. 31, 1950. USDA Agric. Res. Adm., Bur. Entomol. Plant Quar., Spokane, Wash. (Mimeogr.)

[2] Bingham, R.T. 1952. Development of blister rust resistant white pine, 1952. Pages 84-90 *in* White pine blister rust control northwestern project Jan. 1 to Dec. 31, 1952. USDA Agric. Res. Adm., Bur. Entomol. and Plant Quar., Spokane, Wash. (Mimeogr.)

[3] Bingham, R.T. 1953. Development of blister rust resistant white pine, 1953. Pages 69-75 *in* White pine blister rust control northwestern project Jan. 1 to Dec. 31, 1953. USDA Agric. Res. Adm., Bur. Entomol. and Plant Quar., Spokane, Wash.

[4] Bingham, R.T. 1954. Development of blister rust resistant white pine, 1954. Pages 67-70 *in* White pine blister rust control calendar year 1954. USDA For. Serv. Reg. 1, Missoula, Mont. (Mimeogr.)

Two important points that must be emphasized are the early recognition that very high infection rates were necessary to minimize selection of escapes and that controlled pollination was essential. The importance of the first point was not quantified until 1982 (McDonald and Hoff). Without the controlled pollination, none of the detailed genetics work to be discussed later or the development of an integrated deployment plan to minimize the problem of racial variation in the rust (McDonald et al. 1990) would have been possible.

By 1955 the breeding program had settled into a routine, and significant positive results from the artificial inoculations were appearing[5]. Each year's cycle of inoculated progeny was being planted at two locations in Idaho and one in Montana (Bingham 1983). The first progress report of the program was presented to the scientific community (Bingham et al. 1953).

NEW RESEARCH CENTER

In late summer of 1957, the program was at another crossroad. Four cycles of material had accumulated and more were on the horizon. Bingham and his colleague, Tony Squillace, were actively discussing the need for expanded facilities and a breeding arboretum to produce the second generation of resistant pines. Even though the new breeding program was successful and showed much promise, bureaucracies being what they are, little chance was seen to obtain the required funding. At this point another organizational accident happened. Following is a condensation of Bingham's (1983) account of the important event.

One evening in late August 1957, Bingham and his crew were having supper at Clarkia, Idaho, after a day of rust inspection of progenies at the Fernwood, Idaho, site. The supervisor of the local blister rust control force approached Bingham with the news that a group of administrators from the Washington office and Region 1 (Northern Region) of the Forest Service were on a blister rust control inspection trip. The group was running ahead of schedule by a day and wanted to make an unplanned examination of the breeding program. That evening four administrators arrived at Clarkia and the next day was spent at the Fernwood plot. After a thorough scrutiny of the inoculated seedlings, Assistant Chief Bill Swingler is reported to have said, "Well, it looks like you're onto something here. What do you think you should be doing about it?"

Since Bingham and Squillace had already discussed options, Bingham replied with a long list that included a new research facility, new research jobs, and operating funds. In about 2 weeks, notification came that funds were available for the facility. But Bingham had to find free land on which to locate. Dean Ernest Wolletz of the University of Idaho School of Forestry offered a research facility site as well as a location for the needed breeding arboretum. About 1 year later the "Northern Idaho Forest Genetics Center" was in operation (Fig. 5).

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[5] Bingham, R.T. 1955. Development of blister rust resistant white pine, 1955. Pages 10-1 to 10-7 *in* White pine blister rust control calendar year 1955. USDA For. Serv. Reg. 1, Missoula, Mont.

Ultimately, the presence of the Genetics Center and an available arboretum site shifted political and administrative balance to selection of Moscow, Idaho, as the home of one of the Intermountain Forest and Range Experiment Station's (now Intermountain Research Station) Forestry Sciences Laboratories. The laboratory was constructed in 1963 (Fig. 6) and remains in operation today. Thus, Dick's second legacy was the Moscow Forestry Sciences Lab with its national leadership role in forest genetics and silviculture research, root disease and soil productivity research, forest mensuration research, and engineering research on road stability and erosion. Keep in mind, though, this legacy was also more by happenstance than plan. But for an accident in scheduling, this legacy might not be with us today.

NEW STRATEGIES

The next legacy was more planned and has to do with Bingham's role as a research administrator. As mentioned earlier, one of the requests made at the Fernwood meeting was program funding for more research. This wish was also granted. The first new position funded was one seeking to discover chemical markers of resistance. Dr. James Hanover joined the staff of the Genetics Center shortly after completion of the facility in the fall of 1958. He investigated phenolic compounds and monoterpenes until he left the Forest Service for a position at Yale University in 1965. Ray Hoff replaced Hanover in 1965 and for a time continued the search for a chemical marker until this avenue was abandoned for lack of knowledge concerning the nature of resistance and Hoff's research was redirected (Bingham 1983).

In 1966 Bingham was ready to try a new strategy. The specter of racial variation in the rust was much in evidence and many new materials from the controlled breeding were available. A decision was made to investigate the genetics of the host-pathogen couplet.

A new position was created and a second new scientist (Geral McDonald) was added. These moves marked the beginning of our concentrated effort to understand the genetics of resistance and virulence in the rust system. First, we attempted to gain better genetic control of the system by clonal propagation of the pine. We made some progress but not enough to be of experimental use (McDonald and Hoff 1970a). In the meantime, we began construction of symptomologic and genetic definitions of resistance mechanisms by detailed study (Fig. 7) of artificially inoculated, controlled-cross progeny from the large-scale testing program.

These investigations almost immediately encountered opposition from Bingham because he had already concluded that "his" resistance was polygenic and our results were demonstrating a complex association of resistance mechanisms inherited as simple recessive or non-dominant genes (McDonald and Hoff 1970b; Hoff and McDonald 1971; McDonald and Hoff 1971; Hoff and McDonald 1972; Hoff and McDonald 1980). In addition, this avenue was demonstrating the possible functioning of a typical gene-for-gene association in the blister rust pathosystem (McDonald and Hoff 1975; McDonald 1978).

Even though Bingham was sure he was working with a polygenic system, he continued to support us. Thus, Bingham's tolerance for another point of view (perhaps he remembered his own situation from 20 years earlier) encouraged two young scientists to play out their hands, make a contribution to understanding tree diseases, and add another layer of flexibility to the program that should

Figure 5. Appearance of the North Idaho Forest Genetics Center of the USDA Forest Service, Intermountain Forest and Research Station, in October, 1958 (U.S. Forest Service photo).

Figure 6. The Moscow, Idaho, Forestry Sciences Laboratory of the USDA Forest Service, Intermountain Research Station, in 1965 (U.S. Forest Service photo).

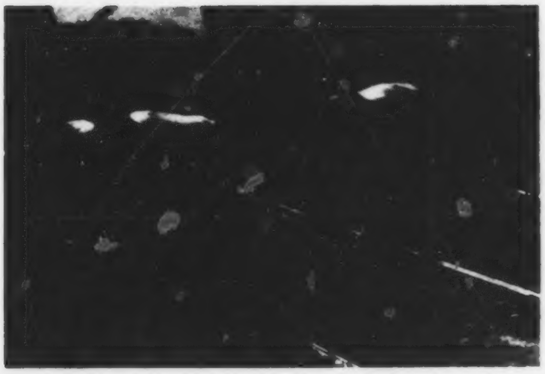

Figure 7. Detailed symptom inspection of a portion of the 50 000 control-pollinated seedlings of western white pine artificially inoculated with the blister rust fungus in September 1964 (U.S. Forest Service photo).

ultimately lead to the successful deployment of a system capable of delivering stable rust management (McDonald et al. 1990). So, Bingham's third legacy was the cultivation of two enthusiastic and committed scientists dedicated to the realization of his ultimate goal--the restoration of western white pine in the mix of species available to the silviculturist.

A LASTING LEGACY

Bingham's fourth legacy is the very wide range of different kinds of parents and progeny he located or planted, mapped, and labeled so that they remain available and suitable for future scientific inquiry. This material will facilitate monitoring for rust races, supply populations for selecting new traits, and provide the foundation for construction, calibration, and validation of integrated management systems of the future (Gerhold et al. 1986; Hagle et al. 1989; McDonald et al. 1990).

The early plantations among these materials have performed so well that they prove in hindsight the level of resistance obtained from the very first was much higher and more durable than anticipated. Perhaps Bingham's most important legacy is the negative impact of the absence of thousands of acres of productive white pine plantations composed of relatively broadly based (genetically) moderately resistant materials. Was Bingham too conservative in interpreting the robustness of the selected populations?

We stand at another crossroad poised to apply a stabilizing integrated management program that can capitalize on 80 years of very sound research, which has produced the most complete understanding of any forest tree disease. Successful application does, however, require some further effort. We need to continue investigation of inoculation processes at all levels until they are understood well enough to produce consistent and repeatable results; we need a program to monitor rust races; we need to continue breeding for resistance for moderate hazard areas such as western Oregon and Washington for western white pine, and southwestern Oregon and northern California for sugar pine; and we need to continue development, verification, and calibration of epidemiological models.

Dick Bingham appears to have been the person for the blister rust research job. Someone was needed who possessed broad interests and experience in all facets of the disease as well as the energy, enthusiasm, and dedication to see a long-term program through. Certainly, much criticism of many aspects of the program could be made (and most of it has been) but any negative observations seem petty now in light of accomplishments made under Bingham's very able guidance.

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OBSERVATIONS ON THE SPERMOGONIAL STAGE OF THE WESTERN GALL RUST FUNGUS

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Neither the life cycle nor the taxonomic status of the fungus causing western gall rust of pines is clearly established. The name is in question because the nuclear events that occur during haploidization from dikaryotic spores to haploid mycelium are in question (Epstein and Buurlage 1988; Hiratsuka 1988). The mechanism of dikaryotization is not known (Epstein and Buurlage 1988). The related question of whether the fungus is homothallic or heterothallic has not been resolved, although some populations appear to be homothallic (Tuskan and Walla 1989). Questions remain regarding the occurrence of a *Cronartium* stage if the name *P. harknessii* is accepted; infection of alternate hosts has been reported (Peterson 1967).

The name *Peridermium harknessii* J.P. Moore [≡*Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka] is used in this paper without claim as to the mechanism of haploidization. The species as currently defined is quite variable so more than one type of nuclear cycle may occur. Nuclear events during a particular stage in the life cycle may characterize or determine the subsequent nuclear cycle. For instance, spermogonia might play a role in dikaryotization of *P. harknessii*. The process of dikaryotization may determine whether the subsequent thallus will be homozygous or heterozygous, whether haploidization will be through disassociation of apparently dikaryotic nuclei or by meiosis, and whether a *Cronartium* stage will occur.

Recent literature indicates that spermogonia of *P. harknessii* are rare or absent and possibly nonfunctional (Epstein and Buurlage 1988; Hiratsuka and Powell 1976; Ziller 1974). In contrast, older literature indicates that the occurrence of *P. harknessii* spermogonia is widespread in North America. They have been reported from Idaho, Montana and Oregon (Weir and Hubert 1917b; Weir, in Peterson 1959), California (Gill 1932; Meinecke 1916; Nelson 1970), New York (True 1938), and Connecticut (from inoculations using aeciospores from California) (Boyce 1957) in the United States, and from British Columbia (van der Kamp, in Hiratsuka and Powell 1976) in Canada. Spermogonia have been associated with each of the previously separated species and forms now considered to cause western gall rust (Table 1). Spermogonia have been observed on galls on a number of pine hosts at various times of the year and at various levels of abundance (Table 1, 2). Not all of these reports demonstrated that galls with spermogonia were actually caused by *P. harknessii*. Particularly suspect are reports that included successful inoculations of alternate hosts or research conducted in areas where *C. quercuum* (Berk.) Miyabe ex Shirai occurs. Some reports of spermogonia are from a period when inoculation of alternate hosts was more commonly successful (Meinecke 1920; Weir and Hubert 1917a). Such reports raise the question of whether spermogonia were seen on galls of the autoecious *P. harknessii* or on galls of *C.*

Table 1. Reports of *Peridermium harknessii* spermogonia

Reporter[a]	Year reported	Spermogonial abundance	*Peridermium* reported	*Pinus* hosts
Meinecke	1916	Rare	*harknessii*	*jeffreyi*
Weir & Hubert	1917	Abundant	*harknessii*	*ponderosa, contorta, attenuata*
Gill	1932	Rare	*cerebroides*	*attenuata, radiata*
True	1938	Common	Woodgate	*sylvestris*
Boyce	1957	Sparse	*harknessii*	*sylvestris, ponderosa*
Nelson	1971	Rare	*harknessii*	*radiata*
van der Kamp	1976	--	*harknessii*	--

[a] References in text.

Table 2. Spermogonial characteristics of *Peridermium harknessii*

Reporter[a]	Month spermogonia observed	Spermogonial stroma position	Aecial production delay	Spermatia	
				Shape	Size (µm)
Meinecke	April	In or under bark	--	--	--
Weir & Hubert	April	Subepidermal	8-16 days	Mostly spherical	2.5 × 2.5
Gill	Jan., Feb.	Immediately below periderm	Few days	Ovate to pyriform	1.7 × 3.0
True	May	Beneath periderm	1 year	--	--
Boyce	April	--	3 days	--	--
van der Kamp	Late fall	--	--	--	--
Walla et al.	May	Between periderm and cortex	Few days	Ovoid to pyriform	1.9 × 3.0

[a] References in text.

quercuum or an undescribed *Cronartium*. Questions remain as to whether spermogonia reported on *P. harknessii* galls are functional and, if so, whether a heterozygous organism can thus be formed.

In 1989, spermogonia were discovered on pine rust galls thought to be caused by *P. harknessii* at two locations in North Dakota. Spermogonia of *P. harknessii* had not previously been observed in this region. In this paper, we characterize the spermogonia noted in North Dakota and compare them with previous reports. We also examine whether the galls with spermogonia were caused by the autoecious *P. harknessii* and whether the occurrence of spermogonia could indicate that subsequent aeciospores are heterozygous.

MATERIALS AND METHODS

More than 1000 galls of western gall rust on ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) were observed in the field in May 1989. Some of those galls were gathered for aeciospore collection from four sites: 1) Horning Tree Farm, Plattsmouth, Nebraska--May 4-5, about 25 galls; 2) Towner Nursery, Towner, North Dakota (ND)--May 10, 4 galls; 3) badlands, Slope Co., ND--May 17-18, about 150 galls; and 4) Denbigh Experimental Forest, Denbigh, ND--May 19, about 75 galls. All collections were made before aecia released spores. Galls were placed in bags and stored in coolers with ice to allow aecial development in a cool humid environment. Galls were observed, primarily to examine development of aecia, one day after gall collection from site 1, one to ten days after gall collection from site 2, two to eleven days after gall collection from site 3, and one to nine days after gall collection from site 4. The maximum number of days galls were examined after collection reflects the length of time until aecia matured. Galls with conspicuous spermatial exudate were placed in individual plastic bags, labeled and stored at 4°C.

After spermatial exudate was noted on one gall from site 2, two other branches from site 2, each with a five-year-old gall, were placed with severed ends in water and covered with plastic bags. One of the branches was kept at 4°C and the other branch was placed on a laboratory bench at about 24°C.

Small droplets of spermatial exudate were transferred to microscope slides and stained with cotton blue in lactophenol. Thirty-three spores from six galls were measured under oil at 1000× to obtain spore dimensions. Spermatia were placed in a vital stain Evans blue (0.5% in water) to determine spore viability. Spermogonia were removed from galls for histology by cutting a block of tissue from the gall around each spermogonium. Samples were prepared for scanning electron microscopy using a modification of the procedure described by Brown and Brotzman (1976). Specimens were fixed in buffered (Millonig's, pH 7.6) 2% osmium tetroxide containing 0.05% Kodak Photo-Flow 200 for 3.5 h at 4°C. Photo-Flow was added to increase wettability of the specimen surface. Following the initial fixation, the samples were washed with five changes of distilled water and then placed in a saturated solution of thiocarbohydrazide for 2.5 h. The samples were washed again with five changes of distilled water and refixed overnight at 4°C in the osmium solution. Following five washes with distilled water, the specimens were dehydrated in an ethanol series and critical point dried using CO_2 as a transitional fluid. The specimens were sputter coated with gold, examined and photographed using a JEOL JSM-35 scanning electron microscope. For light microscopy, gall tissue was fixed in FAA, embedded in paraffin, and sectioned with a rotary microtome. Sections were stained with safranin-fast green following the schedule of Allen (1984).

Aeciospores from three galls with spermatial exudate from site 4 were stored in an ultrafreezer (-74°C) for later use. Aeciospore dimensions and germination characteristics were determined

30 h after sowing spores on 2% water agar. Dimensions of 25 aeciospores were measured using spores from two of these galls. Germination characteristics were determined by counting the number of spores germinated out of 100 and measuring the length, branching characters and percentage of tip lysis of 25 germ tubes of spores from all three galls. Germination characteristics were compared with those previously used to differentiate *P. harknessii* from other rusts (Anderson and French 1965). Aeciospores from each gall were used to inoculate current-year shoots on three-year-old potted seedlings and primary growth on 45- and 59-day-old container-grown seedlings of ponderosa pine.

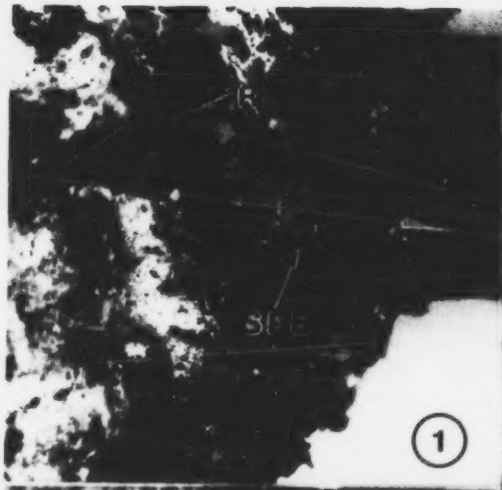
Isolation of the fungus into pure culture was attempted by placing spermatia, sections of spermogonia and sections of gall tissue from galls that had spermogonia onto a defined medium (Allen et al. 1988). One isolate grown in pure culture from a section of gall tissue from a site 2 gall that had spermogonia and one aeciospore sample from a site 4 gall that had spermogonia were analyzed electrophoretically and isozymes were compared with those from isolates of standard *P. harknessii* cultures and spore samples (Tuskan and Walla 1989).

RESULTS

Spermatial exudate was not observed on the 1000+ galls examined in the field, but was observed on seven of about 254 galls gathered for aeciospore collection. One droplet of spermatial exudate (Fig. 1, 2) was noted on May 11 on a five-year-old gall collected the previous day from site 2. Several more droplets of spermatial exudate formed on that gall by May 13 after additional storage at 4°C. Spermatial exudate was also observed May 13 on another gall from site 2, the branch of which was incubated with its lower end in water on the lab bench. Spermatial exudate was observed after two to nine days storage on five of about 75 galls collected at site 4. Spermatial exudate was not found on galls from sites 1 or 3. Several galls from sites 2, 3 and 4 were dissected to look for spermogonia after 18 to 27 days storage but none were found. By that time, aecia were mature and fungal contaminants had overgrown the galls, so that any spermogonia present would likely have been obscured.

Spermatial exudate occurred as a viscous fluid above cracks in the periderm of galls (Fig. 2). The identification of spermatial exudate was confirmed by microscopic observations. Spermatial exudate on freshly collected galls was orange, similar to the color of aeciospores. Spermatial exudate on galls stored for several days was dark orange to black due to the growth of other fungi in the exudate. Resin droplets were common on galls and sometimes appeared similar in size, shape and color to spermatial exudate (Fig. 1). Although resin droplets were colorless, they appeared similar in color to the gall surface on which they were found. The thick, sticky resin droplets retained their shape and hardened upon drying, while the viscous spermatial exudate dried down onto the gall surface or was absorbed into the gall. Spermatial exudate was observed on various areas of galls, including the edges next to the bark collar, sunken areas and nondescript portions of galls. The number of droplets of spermatial exudate per gall ranged from one to more than ten.

Spermogonia formed from hyphal masses near the gall cortex surface. The spermogonial stroma was between the gall cortex and periderm (Fig. 3-5, 8, 9). Spermogonial stromata were indeterminate in size and shape. Margins of spermogonia were not raised so they could not be detected prior to exudate formation without dissection. The spermogonial hymenium was usually, but not always, flat (Fig. 3, 4). It appeared that the hymenium shape was a function of the cortex surface. Aecia originated within the cortex of gall tissue, deeper than spermogonia. Aecia with immature spores were close (within 100 µm) to some spermogonia (Fig. 3). The spermogonial surface was a shiny bright orange color when the overlying periderm was removed from freshly collected galls. The spermogonial surface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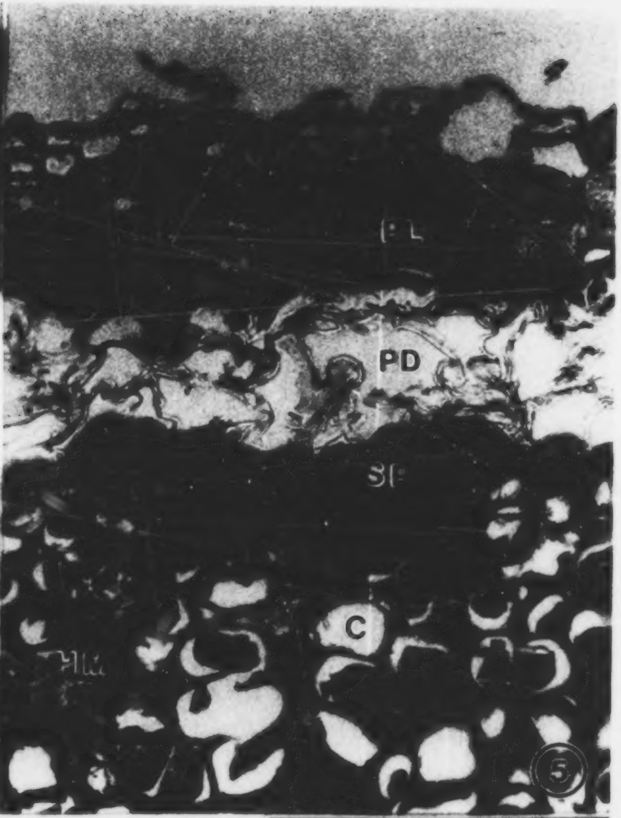
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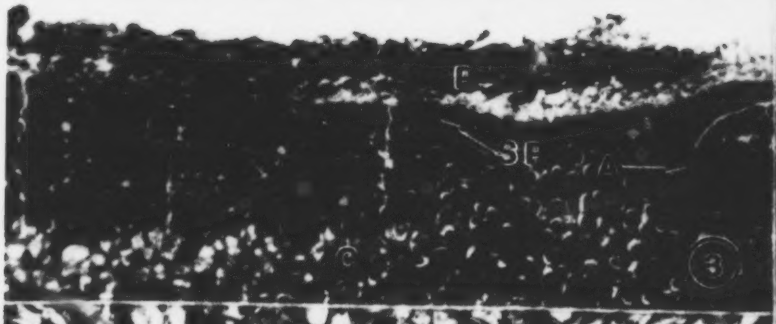
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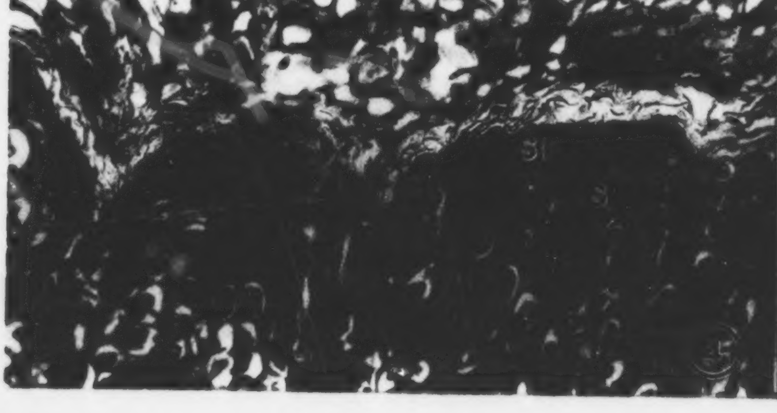
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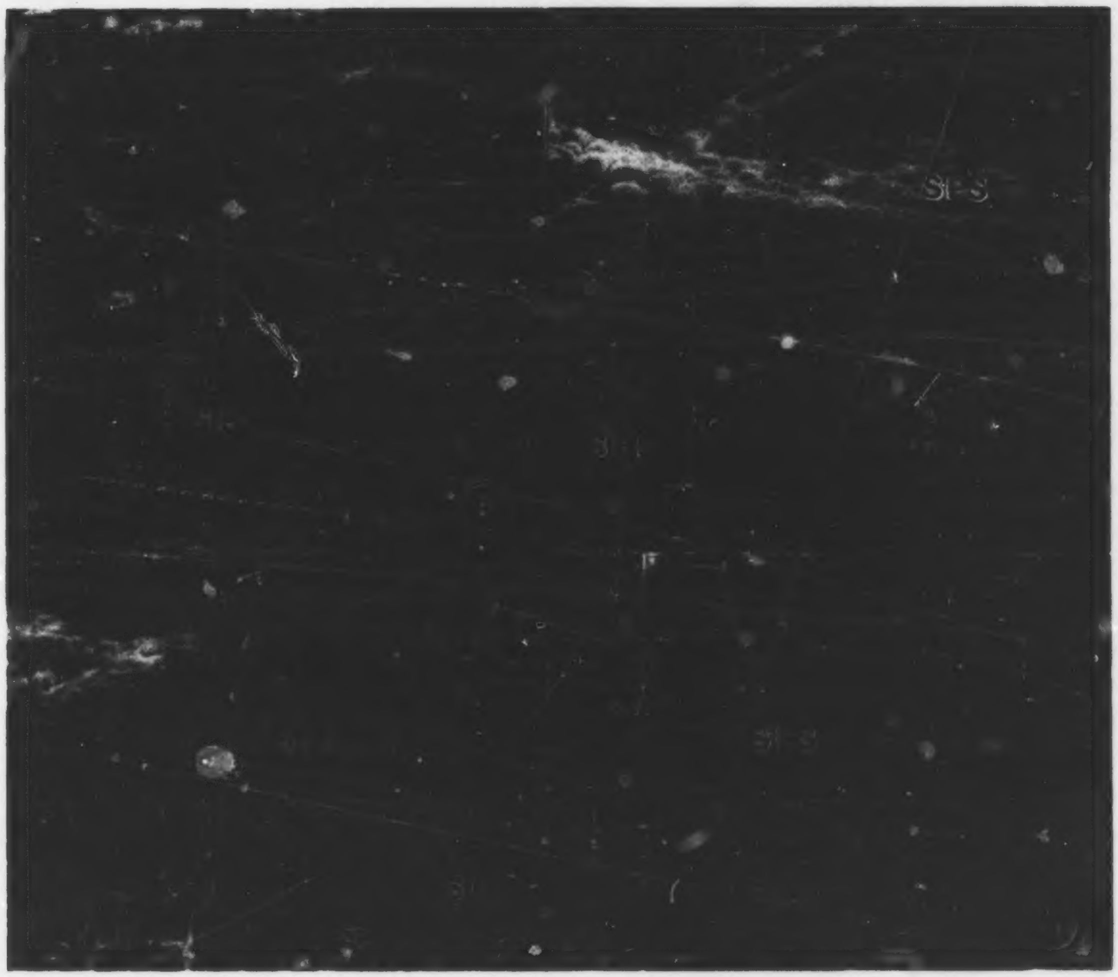

Figures 1-11. **1.** Spermatial exudate (SPE) and resin droplet (R) on *P. harknessii* gall. **2.** Spermatial exudate (SPE) and crack (CR) in periderm from which exudate oozed. **3.** Long, indeterminate spermogonial stroma (SP) between periderm (P) and cortex (C) and immature aecium (A) in cortex (× 55). **4.** Spermogonial stroma (SP) shaped to contour of the cortex surface (CS) (× 150). **5.** Spermogonial stroma (SP) below phellem (PL) and phelloderm (PD) and above cortex (C). Intercellular spaces in cortex are filled with masses of hyphae (HM) (× 300). **6.** Ovoid to pyriform spermatiospores, in water (× 1550). **7.** Spermatiospores mostly ovoid to pyriform, with range of dimensions, in water (× 1550). **8.** Spermogonial stroma (SP) on cortex (C) and below periderm. Periderm is split into lower phelloderm (PD) and upper phellem (PL) (× 92). (Scanning electron micrograph = SEM.) **9.** Spermogonial stroma with base closely associated with cortex (C). Spermatiophores (SPP) are relatively straight and unbranched. Some spermatiophore initials (SPI) are branched (× 1300 SEM). **10.** Ovoid to pyriform spermatiospores (SPS) accumulated above spermatiophores (SPP). Some spermatiophores are releasing spores (arrow) (× 3000 SEM). **11.** Spermatiospore (SPS) partly released from a spermatiophore (SPP). Arrow shows tip of spermatiospore (× 7300 SEM).

became dull orange to brown in color after a few hours exposure or if galls had been stored for several days.

Spermatia were nearly hyaline, ovoid to pyriform (Fig. 6, 7, 10), and 1.4-2.4 μm × 2.1-4.1 μm (mean 1.9 × 3.0 μm) in dimension. Spermatia were not stained by Evans blue, indicating the spores were viable. Spermatiophores were nearly hyaline, nonbranched, slightly tapered, phialidic, 12.3-22.3 μm long and 1.1-2.3 μm wide (Fig. 9-11).

Aeciospore dimensions, spore germination characteristics, isozyme electromorphs and ability to reinfect pine conformed to published characters of *P. harknessii* (Anderson and French 1965; Tuskan and Walla 1989). Orange callus-like cultures similar to those obtained previously from galls of *P. harknessii* were obtained from gall tissue of three galls, one from site 2 and two from site 4. Axenic cultures were not obtained from spermatia or sections of spermogonia. Isozyme analysis indicated the spores and cultures were homozygous. Pines were successfully inoculated with aeciospores from galls with spermogonia, indicating the fungus in those galls was autoecious.

DISCUSSION

The low frequency at which spermatial exudate was found gives the impression that spermogonia are uncommon. However, spermatial exudate of *P. harknessii* had not previously been observed by these authors and was discovered during investigations designed for other purposes. Improved procedures by investigators familiar with spermogonial characteristics would give a more reliable indication of the abundance of spermogonia. Also, the absence of exudate does not mean that spermogonia are not present. What may be rare, or rarely observed, is the development of spermatial exudate. Because we found exudate only under cool humid conditions on galls with immature aecia, similar conditions may favor the development of spermatial exudate *in vitro*. Such conditions may occur after a rain shortly before aecia mature. Spermatial exudate may occur intermittently because spring rain is intermittent in much of the area that western gall rust occurs. Observation of spermatial exudate may be uncommon because of a tendency to make observations and collections after foliage has dried. These points argue that infrequent observation of spermogonia or spermatial exudate may reflect the developmental nature of that stage rather than its frequency. The potential abundance of spermogonia is indicated by reports of Weir and Hubert (1917b) and True (1938). Another indication that spermogonia may be common but not noticed was found during field observations of aecial development at site 3. A portion of the periderm was removed from a number of galls. Later, when we became more familiar with spermogonia in the laboratory, it was realized that similar structures had been observed at site 3 on a number of galls. By that time, the field observations could not be substantiated so we can only speculate that those structures were spermogonia. In any case, spermogonia appear to be more common than is recently reported.

Characters of spermogonia observed in North Dakota were generally similar to previous descriptions of spermogonia of fungi currently considered to be *P. harknessii* (Table 2). Notable exceptions from ours and other observations include van der Kamp's observation of spermogonia in late fall in British Columbia (Hiratsuka and Powell 1976), True's (1938) observation of aecial development one year after spermogonial development, and Weir and Hubert's (1917b) observation of mostly spherical spermatia with average dimensions of 2.5 × 2.5 μm. Except for the spermatial characters noted by Weir and Hubert (1917b), the variable descriptions of spermogonial characters might be explained by different environmental or developmental conditions.

In this study, *P. harknessii* was found to be the causal agent of the galls with spermogonia. Boyce (1957) established that the gall rust he found spermogonia on was autoecious, so it likely was *P. harknessii*. In contrast, questions remain regarding the identity of the fungi in some previous reports of spermogonia on galls reported as those of *P. harknessii*. Persistent reports of successful inoculation of scrophulariaceous hosts with aeciospores from pine galls suggest that there exists a heteroecious pine gall rust that remains undescribed; that may be the fungus associated with spermogonia on some galls reported to be caused by *P. harknessii*. There have also been reports of infection of oaks by inoculation with aeciospores from pine galls in California (Peterson 1967), indicative of *C. quercuum*. Because galls caused by *P. harknessii* and *C. quercuum* f. sp. *banksianae* are morphologically similar and both rusts have been reported in New York and California (Peterson 1967), spermogonia on some galls reportedly caused by *P. harknessii* from those areas may have actually been those of *C. quercuum*, which commonly produces spermogonia.

The potential that spermogonia are common raises the possibility that crosses could be made. Obstacles to making crosses include developing reliable methods to get spermatial exudate to form under controlled situations. Also, the functionality of spermogonia has not been determined. Spermogonia generally function in heterothallic rusts for outcrossing. Among the pine stem rusts, spermogonia have been found to be functional in *C. quercuum* (Yamazaki and Katsuya 1988). The two galls of *P. harknessii* examined here had homozygous isozyme patterns, indicating those thalli had either been self-fertilized or were cross-fertilized with a similar individual. Our previous work (Tuskan and Walla 1989; Walla et al. 1988) found only homozygous individuals. These findings could indicate that 1) spermogonia are not functional, 2) *in vivo* spermatial exudate from spermogonia is rare, or 3) spermatization by insects does not occur in this region. The question of whether spermogonia are functional likely will not be answered until successful crosses are made. Such crosses between identifiable individuals would allow an alternative approach to examining nuclear phenomena in the life cycle of *P. harknessii*, as well as in studies of virulence of this pathogen.

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DESCRIPTION OF TWO COLONY TYPES OF *PERIDERMIUM HARKNESSII*

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Peridermium harknessii (J.P. Moore) was first axenically cultured by Allen et al. (1988) from galls on *Pinus contorta* Dougl. var. *latifolia* Engelm. seedlings. Morphological features of these isolates varied with time. Such variation has been reported with other rusts; for example, Yamaoka and Katsuya (1984) characterized six distinct colony types of *Melampsora* spp. on the basis of macroscopic and microscopic features of colonies, the occurrence and type of sporulation and the number of nuclei. In this paper, we characterize two colony types of *P. harknessii* isolated from galls on *Pinus ponderosa* Dougl. ex Laws. The temperature and nutrient optima for each type are also reported.

INITIATION OF AXENIC CULTURES

Galls were collected at various times from 15 February to 5 August 1989 in a 25-year-old *P. ponderosa* planting at the Horning State Farm near Plattsmouth, Nebraska. Small pieces (1-2 mm^2) of phloem and cortex tissue with aecia and immature but differentiated aeciospores from surface sterilized galls were used as explants. Explants were plated on the medium in Table 1 (medium concentration levels) and incubated in darkness at 18°C. Only explants collected from 26 April to 23 May yielded rust cultures. This period corresponds to the time immediately prior to and during sporulation.

By 20 days after plating, the immature aeciospores germinated to form white aerial hyphae on the surface of many explants. This type of growth never developed beyond this stage. Subsequently, colony morphology developed in a definite sequence that involved at least two distinct vegetative types. By 45 days, friable orange callus-like growth, referred to as orange mycelium (OM), appeared from beneath the white aerial hyphae. By 60 days after plating, white mycelium (WM) grew as sectors within and at the edges of many OM colonies. The OM colony type was maintained indefinitely if transferred to fresh medium about every 25 days, whereas the WM colony type was maintained without transferring. All colony types grew slowly. Confirmation that OM and WM were both *P. harknessii* was by isozyme analysis (Tuskan and Walla 1989), where all 14 isozymes analyzed were similar for the two colony types and 12 of 14 were similar to those of *P. harknessii* aeciospores from Horning State Farm.

We attempted to develop a culture medium on which *P. harknessii* and *P. ponderosa* could grow mutually well. Several media were tested, most of which included or were modifications of those described by Aitken-Christie (1984), Allen et al. (1988), Jacobi (1982), and Linsmaier and Skoog (1965). All media that were supplemented with peptone, soytone or yeast extract (2 g/L) and sucrose or glucose (15 g/L) supported growth of *P. harknessii*.

Table 1. Concentration of different components used in studying effect of macronutrient, micronutrient and organic level on growth of axenic cultures of *Peridermium harknessii*

	Concentration of media components (mg/L)		
	Low	Medium	High
Macronutrients			
KNO_3	80	240	720
$(NH_4)NO_3$	200	600	1 800
$MgSO_4 \cdot 7H_2O$	100	300	900
$CaCl_2 \cdot 2H_2O$	100	300	900
KH_2PO_4	100	300	900
$FeSO_4 \cdot 7H_2O$	12	36	108
Na_2 EDTA	15	50	150
Micronutrients			
$MnSO_4 \cdot 4H_2O$	10	30	900
$ZnSO_4 \cdot 7H_2O$	3	8	24
$CuSO_4 \cdot 5H_2O$	0.01	0.02	0.06
KI	0.2	0.6	1.8
$CoCl_2 \cdot 6H_2O$	0.02	0.05	0.15
$NaMoO_4 \cdot 2H_2O$	0.1	0.3	0.9
H_3BO_3	3	8	24
Organics			
Sucrose	5 000	15 000	30 000
Glucose	5 000	15 000	30 000
Peptone	1 000	2 000	6 000
Soytone	1 000	2 000	6 000
Vitamins			
Thiamine·HCl	0.8	0.8	0.8
Nicotinic acid	0.3	0.3	0.3
Pyridoxine·HCl	0.3	0.3	0.3
Inositol	100	100	100
Glycine	10	10	10

COLONY TYPES

Orange Mycelium Type

OM colonies had a flat to cerebroid surface topography, smooth to irregularly crenate margin, smooth to sparsely fuzzy surface texture, and orange color similar to *P. harknessii* aeciospores (Fig. 1). These colonies were macroscopically similar to type II colonies of *Melampsora* spp. described and illustrated by Yamaoka and Katsuya (1984). Microscopically, OM colonies were made up mostly of single-celled vesicular cells, which seemed to originate from aeciospore initials on the distal surface of the explant (Fig.

Figure 1. Axenic culture of *Peridermium harknessii* showing the orange mycelium (OM) colony type (× 5 magnification).

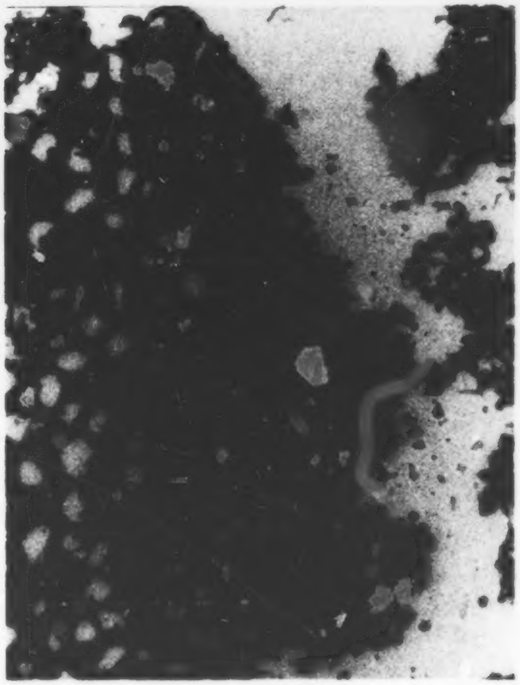

Figure 2. Microscopic cross section of pine host explant (left side) and cells composing OM colonies (right side) (× 400 magnification).

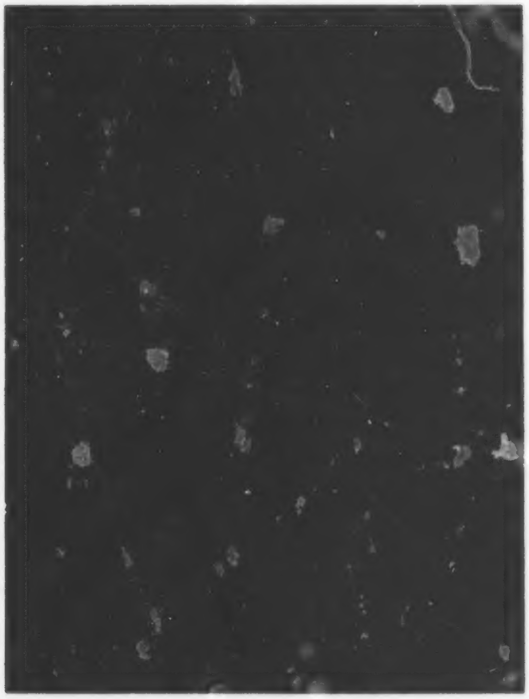

Figure 3. Closeup of two-celled structure with thick-walled hyphae frequently found among single-celled vesicular cells in OM colonies (× 1000 magnification).

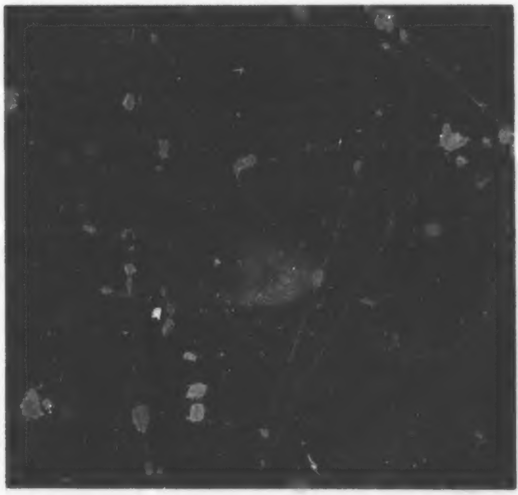

Figure 4. Axenic culture of *Peridermium harknessii* showing the white mycelium (WM) colony type (× 5 magnification).

2). Frequently, two-celled structures were also found from which thick-walled hyphae extended (Fig. 3). Vesicular cells ranged from 4 to 30 µm in diameter. Hyphal strands were irregular in diameter (range 3-9 µm), hyaline, septate and occasionally branched. The larger vesicular cells were similar in size to aeciospores, and contained orange subcellular globules and occasionally had surface ornamentation similar to that of aeciospores. These cells may actually be *de novo* aeciospores that vary in appearance from aeciospores on galls due to the cultural environment. In early stages of colony formation, vesicular cells clustered to form loosely connected friable tissues. With time, these cells were interwoven by thick hyphae and a gelatinous substance to form a firm colony, the center of which was raised above the surface of the medium. Growth into the medium was not observed. Youngest colonies were flat, smooth, orange and friable and contained only vesicular cells. With time, colonies became cerebroid, fuzzy, orange with a gray cast (the underside was brown in the the colony center) and gelatinous. The maturing colonies contained vesicular cells and hyphae. The fuzzy surface texture was due to individual short, thick hyphae growing from many vesicular cells.

White Mycelium Type

WM colonies had a continuous dome-shaped surface topography, smooth margin, fluffy surface texture and cream-white color (tan on the underside) (Fig. 4). These colonies were macroscopically similar to the Type VI colonies of *Melampsora* spp. described and illustrated by Yamaoka and Katsuya (1984). Short thin white aerial hyphae densely covered the surface of the culture. Tightly interwoven thin-walled hyphae occurred below the aerial hyphae. In a cross section through a WM colony, the interior mass of hyphae appeared macroscopically like the context of a mushroom. Hyphae normally grew into the medium across the lower surface of the colony. Hyphae were about 3 µm in diameter, hyaline, septate and occasionally branched. WM colonies appeared to arise as thin-diameter, thin-walled hyphae growing as extensions from the thick-walled hyphae of vesicular cells in OM colonies.

EFFECT OF TEMPERATURE AND NUTRIENTS

OM and WM colonies both grew between a range of 5-30°C (Fig. 5). They differed, however, in that OM grew optimally between 15 and 20°C, whereas WM grew optimally between 20 and 25°C. Temperature had little effect on morphology other than color. At temperatures between 25 and 30°C the center of OM colonies became black after 10 days, and at 10°C, the OM colonies became white.

A broad spectrum experiment (de Fossard et al. 1974), using various concentrations of macronutrients, micronutrients and organic components listed in Table 1, was used to study nutrient optima of OM and WM. A factorial experiment was conducted by preparing various media with each concentration of each component, for a total of 27 different formulas. With OM, higher organic levels caused significantly greater colony weight and diameter growth and decreased cohesiveness between cells regardless of the level of other components (Fig. 6A). Higher macronutrients depressed growth of thick-walled hyphal segment length. In general, changes in micronutrient levels did not influence growth of either colony type. With WM, changes in macronutrient and organic levels of the media did not influence colony growth or form (Fig. 6B).

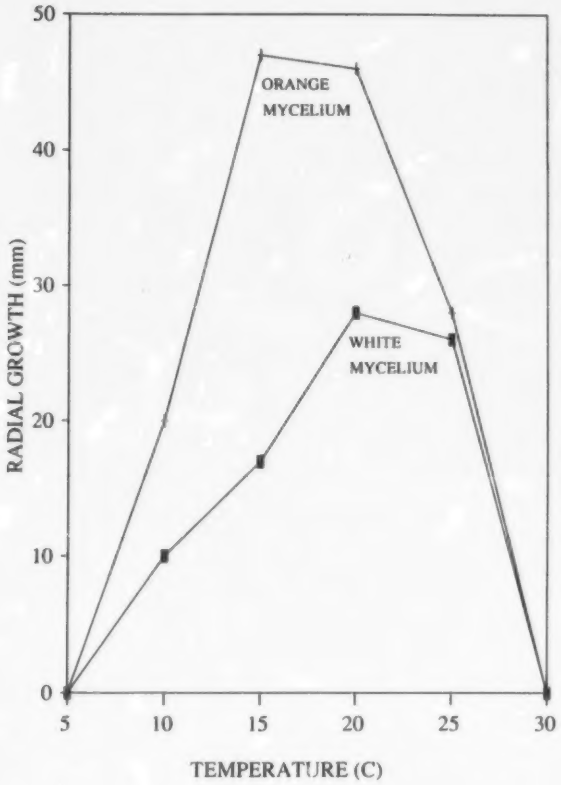

Figure 5. Radial growth of OM and WM colony types at various temperatures.

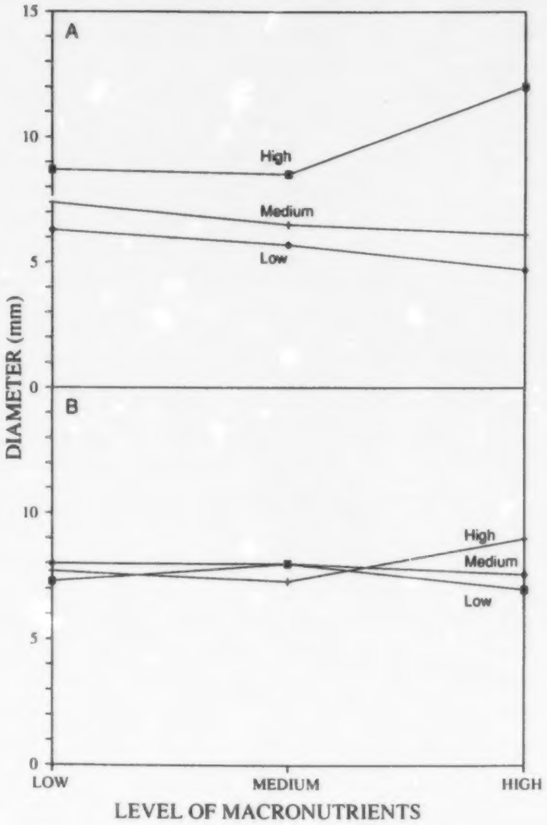

Figure 6. Diameter growth (mm × 10^{-1}) at relative concentration levels of macronutrients and organic nutrients. Lines marked High, Medium and Low correspond to high, medium and low levels of organics, which are defined in Table 1. A. Growth response of OM colonies. B. Growth response of WM colonies.

DISCUSSION AND CONCLUSIONS

In this study, we found that isolation of *P. harknessii* from galls on mature *P. ponderosa* is affected by the time of year galls are collected. During the time just prior to sporulation, isolation success is high on many different media. These isolates subsequently undergo a series of morphological changes into definitive stages. These stages correspond to colony types described previously for other rusts and are similar to colony types that occur in other *Cronartium* spp. (Harvey and Grasham 1974; Hollis et al. 1972; Yamazaki and Katsuya 1987). The common appearance among distantly related rusts is notable.

Macroscopic colony stages reflect the structure and organization of cells at the microscopic level. In the present study, the initial white aerial hyphae are probably germ tubes of aeciospores present when explants were collected. OM colonies appear to grow from a meristematic layer from which aeciospores are generated beneath the white aerial hyphae. Two cell types are found in OM colonies: 1) aeciospore-like cells, many of which develop appendages that resemble rudimentary germ tubes, and 2) two-celled structures with appendages that resemble promycelia of germinating teliospores of *Puccinia* spp. The white mycelium of WM colonies may arise as small diameter hyphae along the length of the appendage of the cells composing the OM. These drastic morphological changes may reflect a change in the nuclear condition. An examination of nuclear development in cells composing OM and WM is now underway in our laboratory.

The life cycle of *P. harknessii* is controversial because a clear, unchallenged description of its nuclear behavior is not available. Most or all previous studies have focused on germinating aeciospores. *In vitro* propagation offers an alternative with which to study nuclear behavior of *P. harknessii*. Development from aeciospore initials to white mycelium may include the stage of nuclear development that is controversial. The use of this axenic system may help define the life cycle of *P. harknessii*.

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MORPHOLOGICAL AND PHYSIOLOGICAL DIFFERENCES IN THE *CRONARTIUM QUERCUUM* COMPLEX

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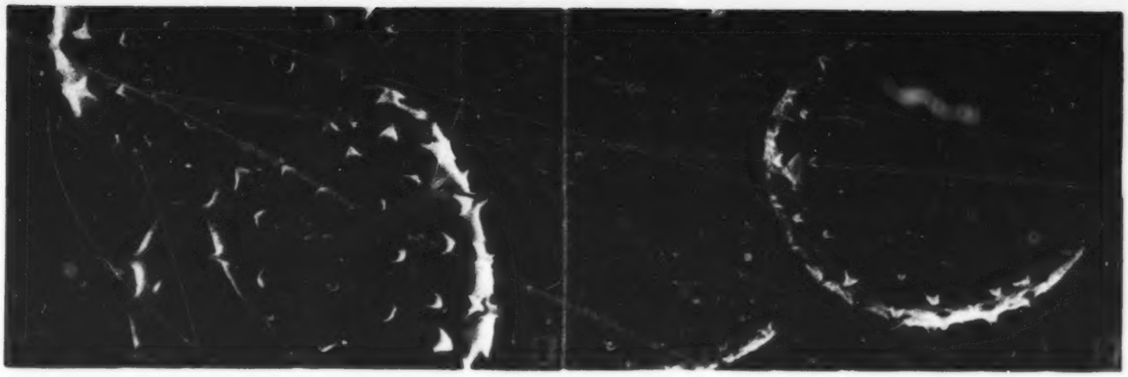
Cronartium quercuum (Berk.) Miyabe is a common and important pathogen of various hard pines in eastern Asia and North America, and has a great capacity to adapt to different hosts and environments. The causal agent of fusiform rust, *C. fusiforme* Hedgc. & Hunt ex Cummins had been recognized as a distinct species. However, Burdsall and Snow (1977) considered *C. fusiforme* to be conspecific with *C. quercuum*, since no consistent morphological differences could be found between the two taxa. They thus treated the former species as a forma specialis of *C. quercuum*.

Between taxa in eastern Asia and North America, some differences have been found in the season that infection of pine occurs and the susceptibility of pine and oak species. To compare various morphological and physiological characteristics of *C. quercuum* from these two areas, studies using either fresh materials or dried specimens from some herbaria have been conducted at Morioka, Japan, and at Athens, Georgia, USA.

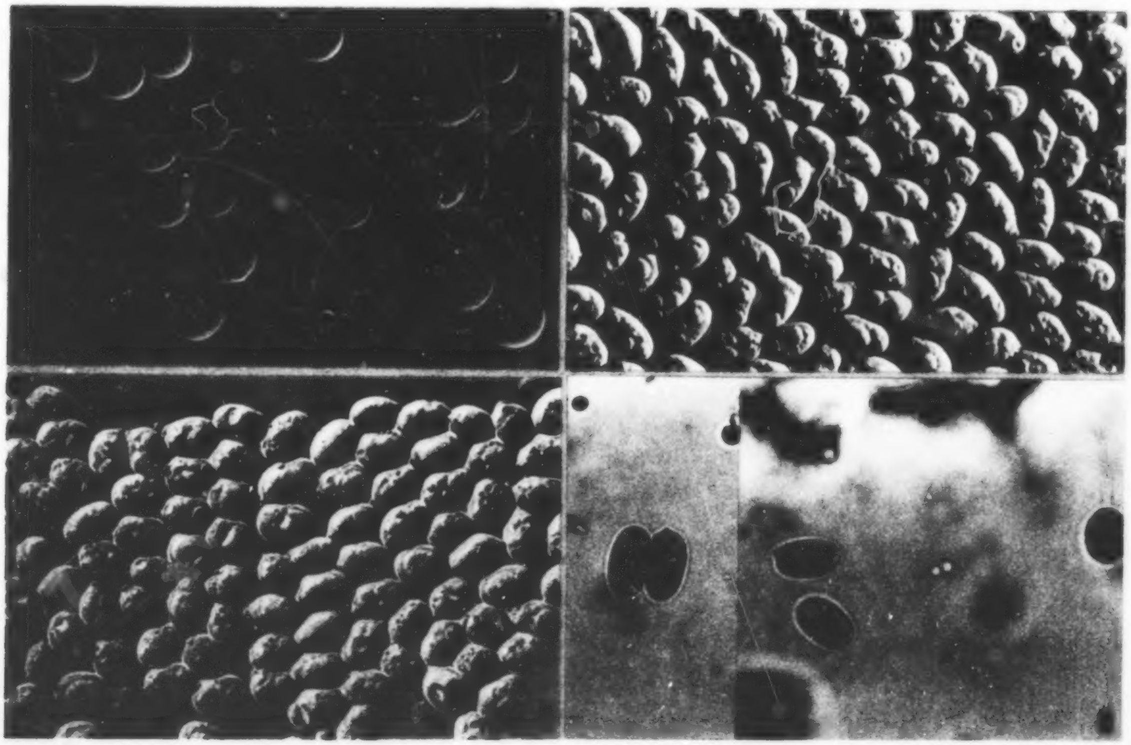
MORPHOLOGY OF UREDINIOSPORES

Urediniospores of *C. quercuum* on various *Quercus* species were compared morphologically using dried specimens from the Arthur Herbarium (PUR) at Purdue University, and the National Mycological Herbarium of Canada (DAOM) as well as fresh spores from Morioka and Athens. Urediniospore size was not affected by geographic source or oak species.

One difference was observed in spore ornamentation between Japanese and North American collections. The urediniospores from North America usually had smaller spines but more per unit area than did the Japanese collections (Fig. 1). This feature appeared stable on *C. quercuum* f. sp. *fusiforme* (Fig. 2). We have not examined collections positively identified as urediniospores of f. sp. *banksianae*, *echinatae* and *virginianae*. However, Grand and Moore (1972) reported no difference between *C. fusiforme* and *C. quercuum* (American pine host unspecified). A detailed comparison should thus be made of the surface characteristics of urediniospores for the Japanese and the three other formae speciales.



Figures 1, 2. Scanning electron micrographs of urediniospores of *Cronartium quercuum*. 1. Japanese isolate on *Quercus mongolica* var. *grosseserrata* (Morioka, Japan). **2.** f. sp. *fusiforme* on *Q. rubra*, by inoculation with aeciospores (Isolate No. M-85).



Figures 3-6. Basidiospores of *Cronartium quercuum*. 3. On *Q. mongolica* var. *grosseserrata* (Morioka, Japan). **4.** f. sp. *virginianae* on *Q. rubra*, by inoculation with aeciospores (Isolate No. MASS-85). **5.** f. sp. *fusiforme* on *Q. rubra*, by inoculation with aeciospores (Isolate No. M-85). **6.** Lectotye on *Q. tinctoria*.

MORPHOLOGY OF BASIDIOSPORES

Basidiospores are usually considered of no significance in the taxonomy of rust fungi. However, in *Coleosporium*, the causal agents of pine needle rusts, and several other groups of rust fungi, basidiospores are considered important at the species level (Cummins 1978; Kaneko 1976, 1981; Kaneko and Hiratsuka 1984). In this study, morphological comparisons were made of 50 basidiospore samples of *C. quercuum* from Japan (Fig. 3) and North America (Fig. 4-6) on various *Quercus* species. Most American collections were obtained by inoculations with aeciospores onto *Quercus rubra* L. The names of the formae speciales of these American collections were known. Lactophenol was used as a mounting fluid for all observations.

When we observed basidiospores cast directly from teliospores on glass slides or which had been concentrated on millipore filters following collection in pH 2 water by Miller's (1970) method, basidiospore morphology was quite stable even if they had been dried on glass slides (Fig. 7). However, when observing dried specimens of telia that had cast basidiospores, basidiospore size was sometimes slightly smaller than that of cast spores since dried specimens contain immature spores still attached to sterigmata. However, length-width ratios for basidiospores discharged from fresh teliospores and those on dried teliospores of the same collection were essentially the same.

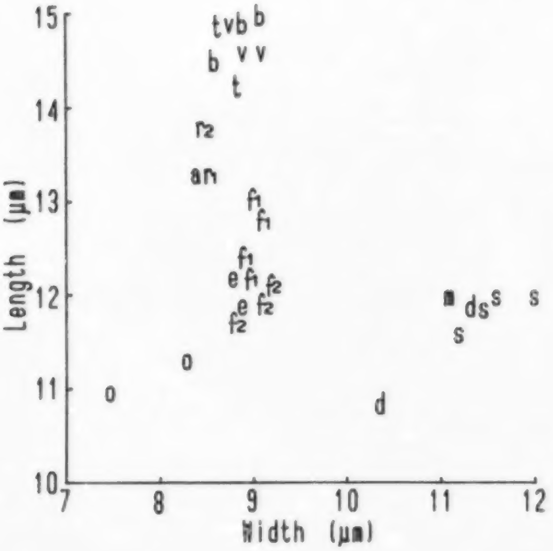


Figure 7. Average dimensions of 50-basidiospore samples of *Cronartium quercuum*. a. Dried specimen on *Quercus marilandica* (Delaware, USA, DAOM 20882). **b.** f. sp. *banksianae* (Isolate No. 692) on *Q. rubra*, by inoculation with aeciospores. **d.** Dried specimen on *Q. serrata* (Morioka, Japan). **e.** f. sp. *echinatae* (Isolate No. 16-68) on *Q. rubra*, by inoculation with aeciospores. **f1.** f. sp. *fusiforme* (Isolate No. M-85) on *Q. rubra*, by inoculation with aeciospores. **f2.** f. sp. *fusiforme* (Isolate No. LCSC-20) on *Q. rubra*, by inoculation with aeciospores. **m.** On *Q. mongolica* var. *grosseserrata* (Morioka, Japan). **o.** Dried specimen of f. sp. *fusiforme* (Isolate No. LCSC-20) on *Q. rubra* by inoculation with aeciospores. **r1.** Dried specimen on *Q. rubra* (Ontario, Canada, DAOM 43990). **r2.** Dried specimen on *Q. rubra* var. *borealis* (Ontario, Canada, DAOM 48677). **s.** On *Q. serrata* (Morioka, Japan). **t.** Lectotype on *Q. tinctoria*. **v.** f. sp. *virginianae* (Isolate No. MASS-85) on *Q. rubra*, by inoculation with aeciospores.

Significant morphological differences were evident between Japanese and North American collections. Basidiospores from Japan were typically globoid in contrast to ellipsoidal or ovoid shape of North American collections. Among the Japanese collections on several *Quercus* species, no significant differences could be found. One difference was found within the American collections. The basidiospores of f. sp. *banksianae* and *virginianae* were distinctly longer than those of f. sp. *fusiforme* and *echinatae*. Slight differences were noted among basidiospores of f. sp. *fusiforme*. Basidiospore shape of the lectotype of *C. quercuum* on *Q. tinctoria* Bartr. (≡ *Q. velutina* Lam.) (Pennsylvania, USA, NYBG Fungus Type Project No. 104) designated by Peterson (1973) was quite similar to that of the f. sp. *banksianae* and *virginianae* group.The basidiospore length from dried specimens of *C. quercuum* on *Q. rubra* and *Q. rubra* var. *borealis* (Michx. f.) Farwell (≡ *Q. rubra*) from Ontario, Canada, and on *Q. marilandica* Muenchh. from Delaware, USA, was intermediate between the f. sp. *banksianae* and *virginianae* group and the f. sp. *fusiforme* and *echinatae* group. The size of mature basidiospores on those *Quercus* species may be similar to that of the f. sp. *banksianae* and *virginianae* group.

The results obtained here show the basidiospore morphology of the *C. quercuum* complex may be separated into at least three distinct groups.

COLOR OF BASIDIOSPORES

We compared the color of basidiospore contents in several ways. Basidiospore masses of the four formae speciales (f. sp.) from the USA concentrated on millipore filters by Miller's method were bright orange. Under a light microscope, the whole basidiospore of each of the four f. sp. was filled with orange-colored oil drops. In contrast, basidiospore masses on millipore filters of Japanese collections on *Q. mongolica* Fisch. var. *grosseserrata* Rehd. et Wils. and *Q. serrata* Thunb. were nearly colorless. Under a light microscope, the whole basidiospore from Japanese collections was observed to be nearly hyaline. This difference was verified with a microscopic spectrophotometer (Olympus AH2-STK). In the Japanese basidiospores, light transmittance was almost 100% for any wavelength (Fig. 8). In the American materials, light transmittance was decreased at 450-500 nm wavelength.

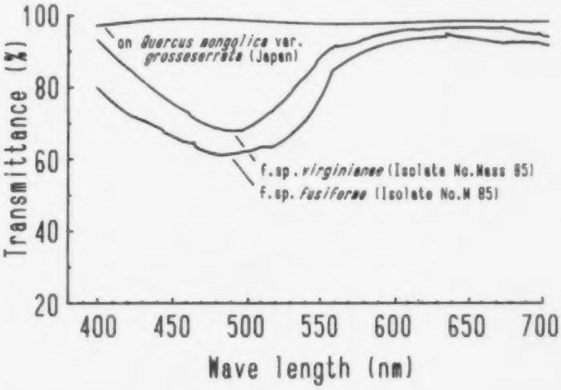


Figure 8. Light transmittance curves of basidiospores of *Cronartium quercuum* measured with a microscopic spectrophotometer.

It is evident from the above that the basidiospore color of the *C. quercuum* differs between Japanese and North American collections.

GERMINATION TYPES OF BASIDIOSPORES

Basidiospores of rust fungi may germinate directly by a thin germ tube or indirectly by the formation of secondary basidiospores. These germination types are influenced by environmental conditions (Bega 1960). To determine whether the effects of certain conditions on germination type for Japanese collections and f. sp. *fusiforme* are similar, several germination experiments were conducted, using telia on *Q. serrata* collected in the experimental forest of Tohoku Research Center, Morioka, and those of f. sp. *fusiforme* on *Q. rubra* inoculated with aeciospores from *Pinus taeda* L. (loblolly pine) in South Carolina (Isolate LCSC-20). All germination tests were done at 20°C in the dark.

When using basidiospores cast directly from telia onto 1.5% water agar on glass slides, indirect germination was noted in 89% and 76% of basidiospores on *Q. serrata* from Japan and of f. sp. *fusiforme*, respectively (Table 1). This difference seems to be insignificant since the rates of direct and indirect germination on water agar vary considerably.

In distilled water, all basidiospores of f. sp. *fusiforme* germinated directly, whereas direct germination occurred in 26% of basidiospores from *Q. serrata* (Table 2).

When basidiospores were placed on 1.5% water agar after being concentrated on a millipore filter following collection in acid water, all the spores of f. sp. *fusiforme* germinated directly. Under the same conditions, many basidiospores from Japan still germinated indirectly though the rate of direct germination increased when the incubation period of telia in a humid petri dish containing acid water was prolonged from 4 hours to 24 hours (Table 3). This suggests that basidiospores of f. sp. *fusiforme* lose completely the ability for indirect germination when they are kept in water for several hours.

When basidiospores were transferred to and allowed to germinate on 1.5% water agar after collecting the spores on dried glass slides by placing the slides under germinating telia for 4 hours in petri dishes, most spores of f. sp. *fusiforme* germinated directly, though direct germination occurred in only 23% of the Japanese collection (Table 4).

Unfortunately, it was necessary to carry out these studies separately to avoid introduction of foreign strains of these pathogens. However, from the above results, it would appear that there is a physiological difference in response to environmental conditions on the germination type of basidiospores between collections of *C. quercuum* in Japan and f. sp. *fusiforme* in the USA.

CONCLUSION

Some morphological and physiological differences have been found in *C. quercuum*. Particularly, the Japanese form on *Quercus* spp. differs from the North American collections in morphology and color of basidiospores and surface characteristics of urediniospores. Additional research on these features will lead to the solution of the taxonomic problem within the *C. quercuum* complex.

Table 1. Germination of basidiospores cast on 1.5% water agar[a]

Rust material	Germ. (%)	Spores counted	Direct (%)	Indirect (%)
C. quercuum on *Q. serrata* from Japan	92	500	11	89
C. quercuum f. sp. *fusiforme* (Isolate No. LCSC-20)	94	1500	24	76

[a] Germinating telia were placed over water agar for 12 h at 20°C.

Table 2. Germination of basidiospores in distilled water[a]

Rust material	Germ. (%)	Spores counted	Direct (%)	Indirect (%)
C. quercuum on *Q. serrata* from Japan	87	500	26	74
C. quercuum f. sp. *fusiforme* (Isolate No. LCSC-20)	97	1000	100	0

[a] Germinating telia were placed over water agar for 12 h at 20°C.

Table 3. Germination of basidiospores collected in pH 2 water, washed in distilled water and allowed to germinate on 1.5% water agar

Rust material	Incubation time of telia on pH 2 water (h)	Germ. (%)	Spores counted	Direct (%)	Indirect (%)
C. quercuum on *Q. serrata*	4	64	204	5	95
from Japan	24	52	500	78	22
C. quercuum f. sp. *fusiforme*	4	92	1000	100	0
(Isolate No. LCSC-20)	24	87	1000	100	0

Table 4. **Germination of basidiospores cast on dried glass slides, transferred to and allowed to germinate on 1.5% water agar[a]**

Rust material	Germ. (%)	Spores counted	Direct (%)	Indirect (%)
C. quercuum on *Q. serrata* from Japan	39	220	23	77
C. quercuum f. sp. *fusiforme* (Isolate No. LCSC-20)	45	752	98	2

[a] Germinating telia were placed on dried glass slides for 4 h at 20°C.

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MORPHOLOGY, CYTOLOGY, AND TAXONOMY OF STEM RUSTS ON FIVE-NEEDLE PINES IN JAPAN

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In Japan, three species of stem rusts, *Cronartium ribicola* J.C. Fischer ex Rabenhorst, *Peridermium yamabense* Saho et I. Takahashi and *Endocronartium sahoanum* Imazu et Kakishima are known to occur on five-needle pines.

Cronartium ribicola is a heteroecious species whose alternate hosts are *Ribes* spp. and *Pedicularis* spp., and is distributed in the mountains in Central Honshu and the lowlands in Hokkaido. Its host in Central Honshu is a native pine, *Pinus pumila* Regel, but the host in Hokkaido is *P. strobus* L. which was introduced from North America long ago (Yokota and Uozumi 1976; Wicker and Yokota 1976; Hama 1987). *Peridermium yamabense* and *E. sahoanum* are both autoecious species on *P. pumila*. The former species is distributed in Hokkaido (Saho 1981), whereas the latter is distributed in Northern Honshu (Imazu et al. 1989) (Fig. 1).

Comparative study on their morphology and nuclear behavior during spore germination was carried out in order to clarify the characteristics of the three rust fungi. In this paper, we summarized the results of our study and discussed the phylogeny of the rusts in Japan.

MORPHOLOGY

The blister symptoms on five-needle pines caused by the three rusts are similar to each other. *Cronartium ribicola* (Fig. 2a) and *E. sahoanum* (Fig. 2c) produce their sori both on twigs and older branches, but *P. yamabense* produces its sori on only 1- to 2-year-old twigs (Fig. 2b).

Sori of three species are pale yellow and similar to peridermium-I of Sato and Sato (1985). The spores of *C. ribicola* are broadly ellipsoid, subglobose or obovoid and 20-32 × 13-23 µm. These walls were hyaline, 2-3 µm thick with annulate warts. The spores of *P. yamabense* are broadly ellipsoid, subglobose or obovoid and 23-40 × 16-31 µm. Walls were hyaline, 2-3 µm thick with longitudinal ditch-like warts. The spores of *E. sahoanum* are broadly ellipsoid, subglobose or obovoid and 24-42 × 17-31 µm. Walls were hyaline, 2-3 µm thick with annulate warts.

The scanning electron microscopic observations showed that *C. ribicola* (Fig. 3) and *E. sahoanum* (Fig. 5) had annulate warts on the spore walls and long warts on the inner surface of the peridial cells. However, *P. yamabense* had longitudinal ditch-like warts on the spore walls and short warts on the inner surface of the peridial cells (Fig. 4).

The length and width of 200-spore samples were measured in order to compare the spore sizes of three species. The spore samples of the species were collected from various locations of Japan. The result is shown in Figure 6. The spores of *P. yamabense* and *E. sahoanum* were larger than those of *C. ribicola* in average dimensions.

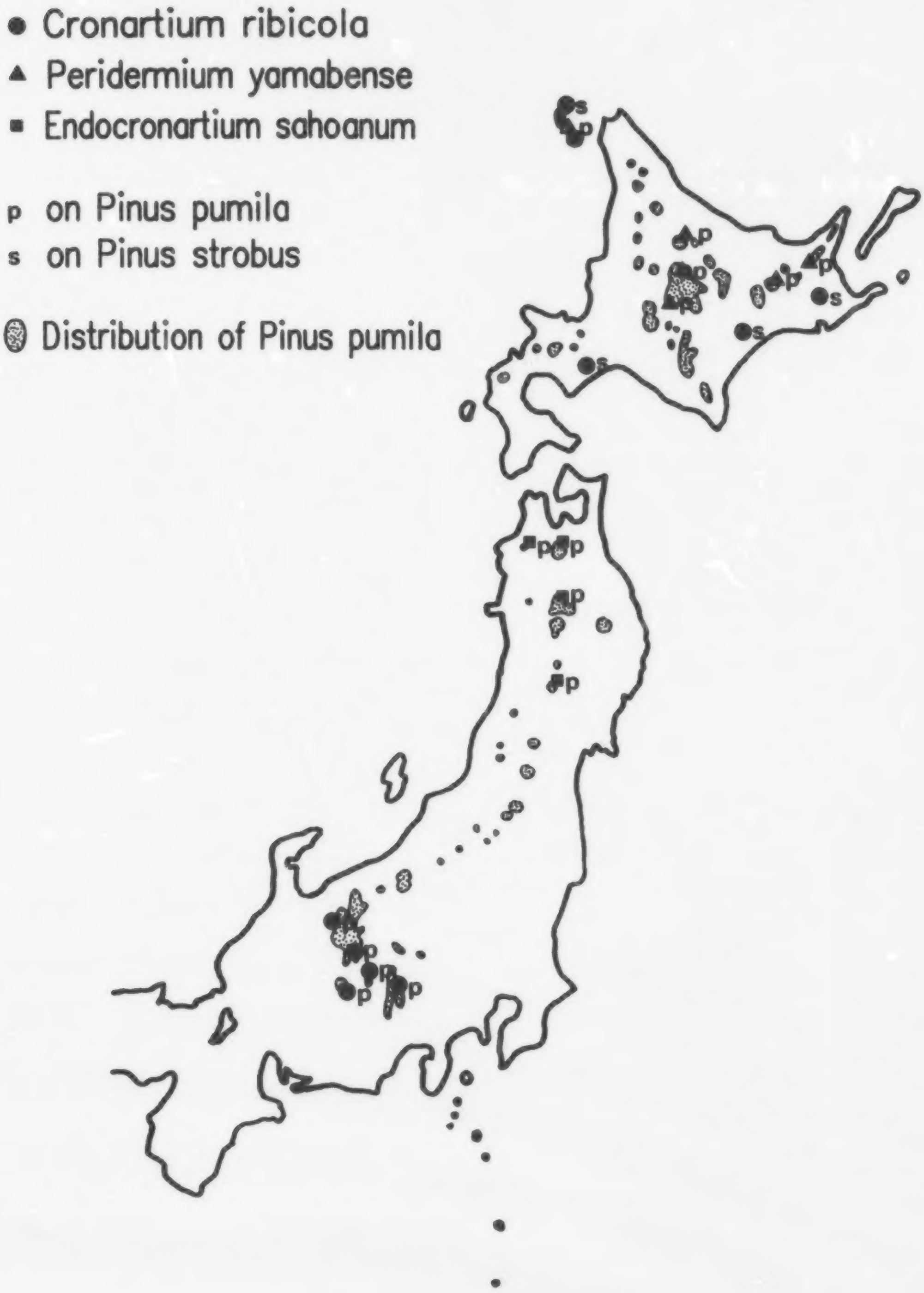

● Cronartium ribicola
▲ Peridermium yamabense
■ Endocronartium sahoanum
p on Pinus pumila
s on Pinus strobus
Distribution of Pinus pumila
s
p
p
p
p
s
s
s
p
p
p
p
p
p
p
p

Figure 1. Geographic distribution of three stem rusts, *Cronartium ribicola*, *Peridermium yamabense* and *Endocronartium sahoanum* in Japan.

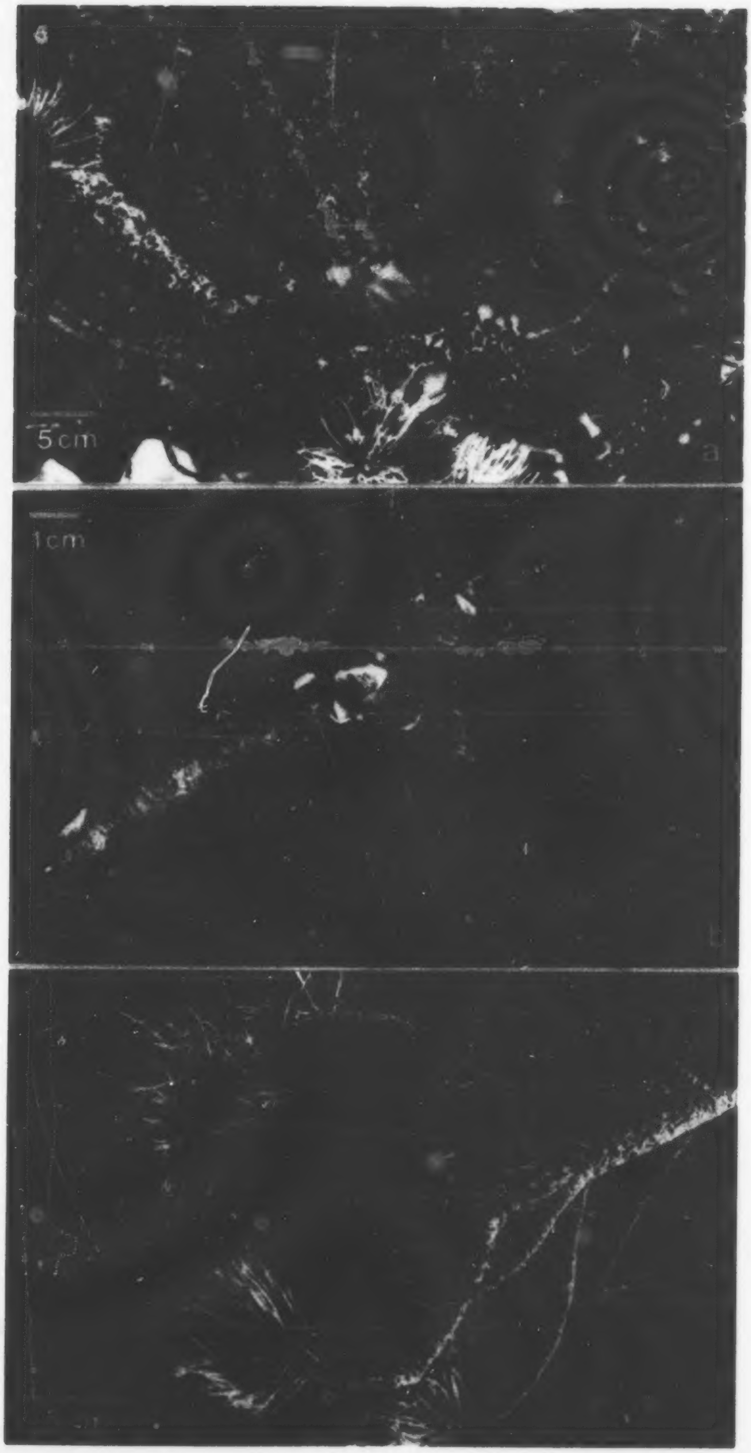


Figure 2. **Symptoms and signs** (sori) **of three stem rusts on the stems of** ***Pinus pumila*****:** **a.** *Cronartium ribicola*; **b.** *Peridermium yamabense*; **c.** *Endocronartium sahoanum.*

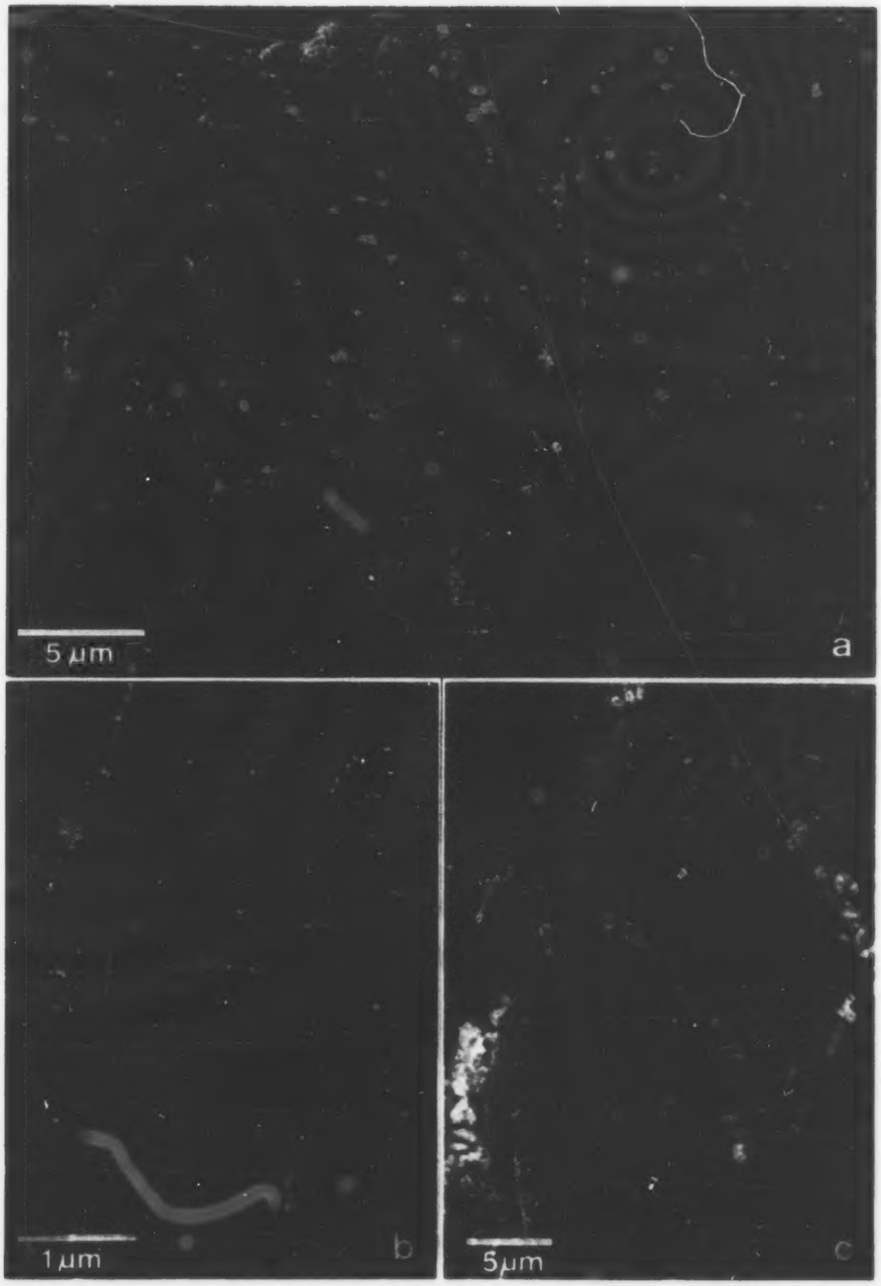


Figure 3. **Surface structures of spores and peridial cells of *Cronartium ribicola* observed by SEM:** **a.** aeciospore; **b.** warts on an aeciospore; **c.** inner surface of peridial cells.

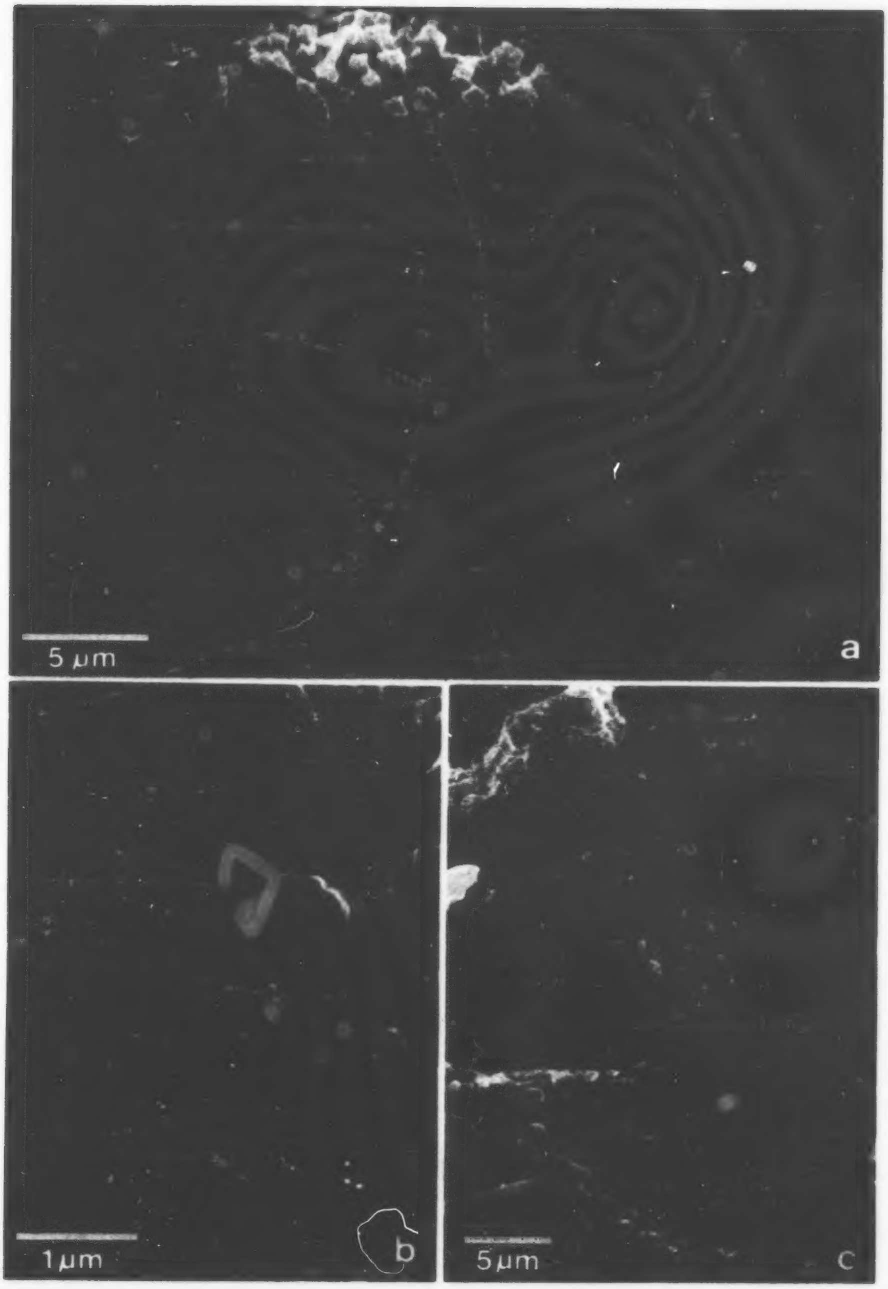


Figure 4. **Surface structures of spores and peridial cells of *Peridermium yamabense* observed by SEM: a.** aeciospore; **b.** warts on an aeciospore; **c.** inner surface of peridial cells.

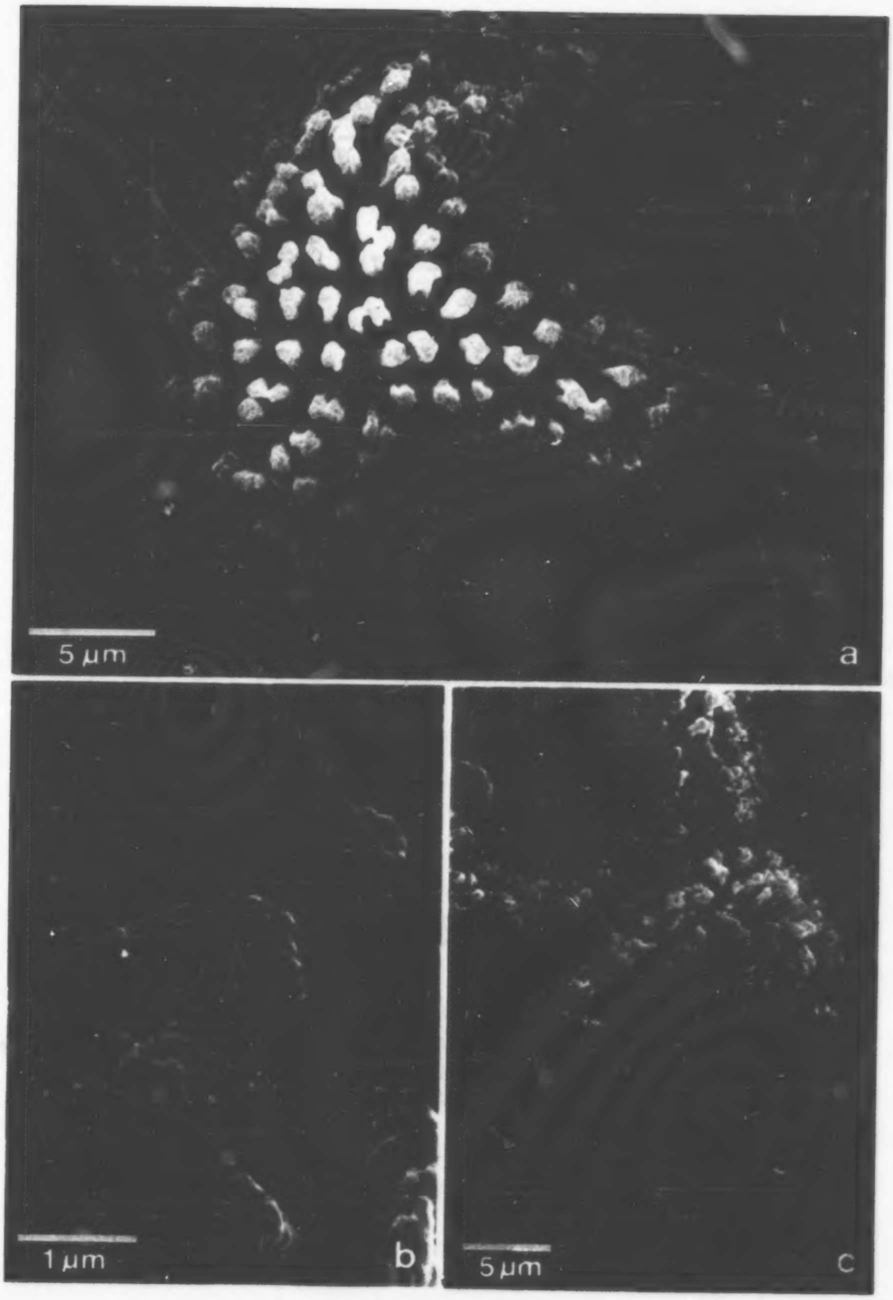


Figure 5. **Surface structures of spores and peridial cells of *Endocronartium sahoanum* observed by SEM:** **a.** teliospore; **b.** warts on a teliospore; **c.** inner surface of peridial cells.

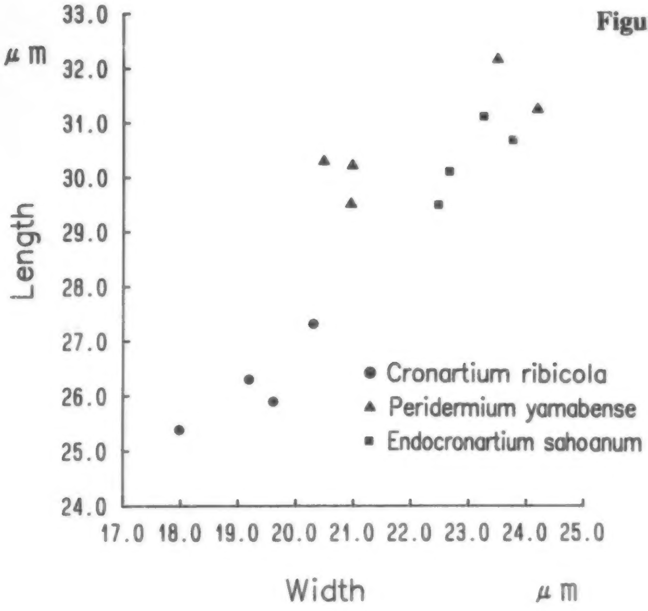


Figure 6. The average dimensions of 200-spore samples. The spore samples of three species were collected from various locations of Japan. Each plot shows the average dimensions of 200 spores collected at one location.

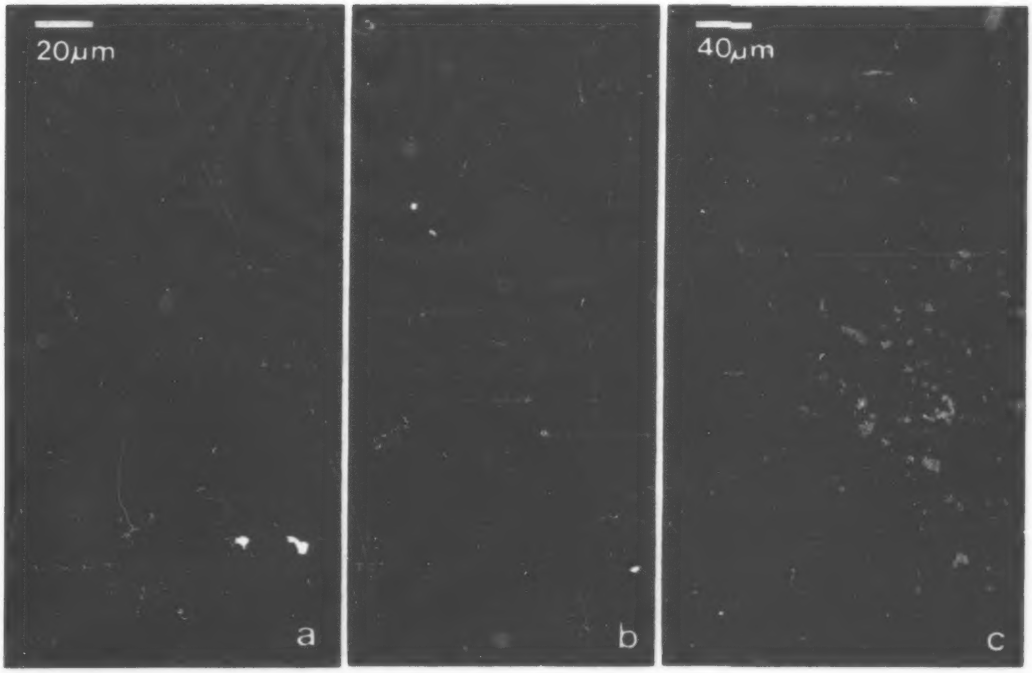


Figure 7. Fluorescence micrographs of nuclei in spores and germ tubes of *Cronartium ribicola*: a. two nuclei in spores and a young germ tube at about 4 h after germination; **b.** two nuclei in a young germ tube at about 6 h; **c.** two nuclei in a germ tube at about 24 h.

CYTOLOGY

For the observation of the nuclear behavior and morphology of the germ tubes during spore germination, the nuclei in the spores and germ tubes were stained with DAPI (4′,6-diamidino-2-phenylindole) and the germ tubes were stained with Calcofluor White M2R (SIGMA Fluorescent Brightener 28) by the method reported previously (Imazu et al. 1989). After staining, they were observed with an epifluorescent microscope.

The number of nuclei in a spore or a germ tube of *C. ribicola* remains constant at two during spore germination (Fig. 7). Its germ tubes produce many branches. Two nuclei were observed in the spores and young germ tubes of *P. yamabense* (Fig. 8a, b). At 16-18 h after germination, the tip of the germ tube began to swell and one nucleus was observed in the tip (Fig. 8c). A swollen vesicle was formed at the tip of the germ tube (Fig. 8d). The vesicle was separated by a septum, and contained four nuclei (Fig. 8d, e). However, as reported by Hiratsuka (1986), occasionally only two nuclei separated by a septum were observed (Fig. 8f). The nuclear behavior and morphology of the germ tubes of *E. sahoanum* were similar to those of *P. yamabense*. Two nuclei were observed in the spores and young germ tubes (Fig. 9a, b). At 16-18 h after germination, the tip of the germ tube often, with some branches, began to swell and one nucleus was observed in the tip (Fig. 9c). A swollen vesicle was formed at the tip of the germ tube and contained four nuclei (Fig. 9d). It was separated by a septum. Four nuclei were also observed in the narrow pointed hypha produced from the vesicle (Fig. 9e). At about 48 h after germination, occasionally a septum was observed in the narrow pointed hyphae (Fig. 9f).

Morphology of germ tubes and nuclear behavior during spore germination of the three species are illustrated in Figure 10. *Cronartium ribicola* was different from *P. yamabense* and *E. sahoanum* in morphology of germ tube and nuclear behavior during spore germination. However, those of *P. yamabense* and *E. sahoanum* are similar to each other.

We consider that nuclear behavior of *E. sahoanum* during spore germination is characterized by karyogamy and meiosis. From our morphological and cytological observations, *P. yamabense* is considered to be a member of *Endocronartium*.

TAXONOMY AND PHYLOGENY

The spore surface structure of *C. ribicola* is very similar to that of *E. sahoanum*, but the spore size of *E. sahoanum* is larger than that of *C. ribicola*. The spore surface structure of *P. yamabense* is apparently different from the two other species. The spore size of *P. yamabense* is similar to that of *E. sahoanum*. On the other hand, the nuclear behavior during spore germination of *P. yamabense* is similar to that of *E. sahoanum* and apparently different from that of *C. ribicola*. Therefore, the three rust fungi are different from each other in morphology and cytology (Table 1).

As described at the beginning, three stem rusts on the native pine, *P. pumila*, are separated by their geographical distribution (Fig. 1). From our field observations, it is supposed that the distribution of the three species is related to the biological and geological conditions of their habitats. Their host, *P. pumila*, is distributed in eastern Siberia, Kamchatka, northeastern China, Korea and Japan (Hotta 1974). In Japan, it is sporadically distributed at high elevations in mountainous areas (Hayashi 1969). We noticed that there was a relationship between the vegetation and the rust species in these habitats. The habitat of *C. ribicola* on *P. pumila* is located in the alpine zone in Central Honshu. This habitat is rich in plant species, and *Pedicularis yezoensis* Maxim., which is an alternate host of *C. ribicola*, often grows around

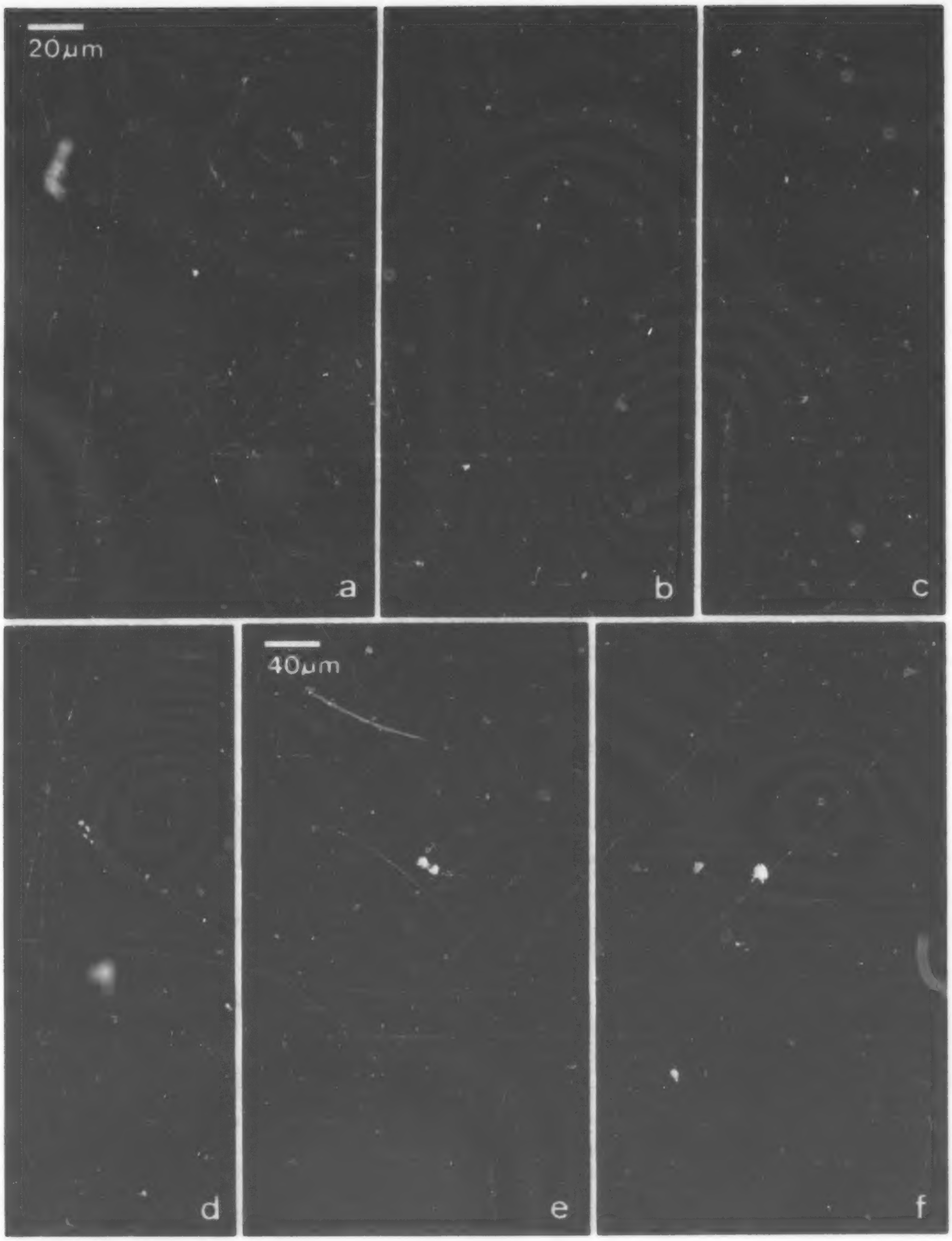


Figure 8. Fluorescence micrographs of nuclei in spores and germ tubes of *Peridermium yamabense*: **a.** two nuclei in spores; **b.** two nuclei in a young germ tube at about 6 h after germination; **c.** single nucleus in the tip of a germ tube at about 16 h; **d-e.** four nuclei in a vesicle at the tip of a germ tube at about 24 h; **f.** a germ tube separated by a septum at about 24 h, each cell contains one nucleus.

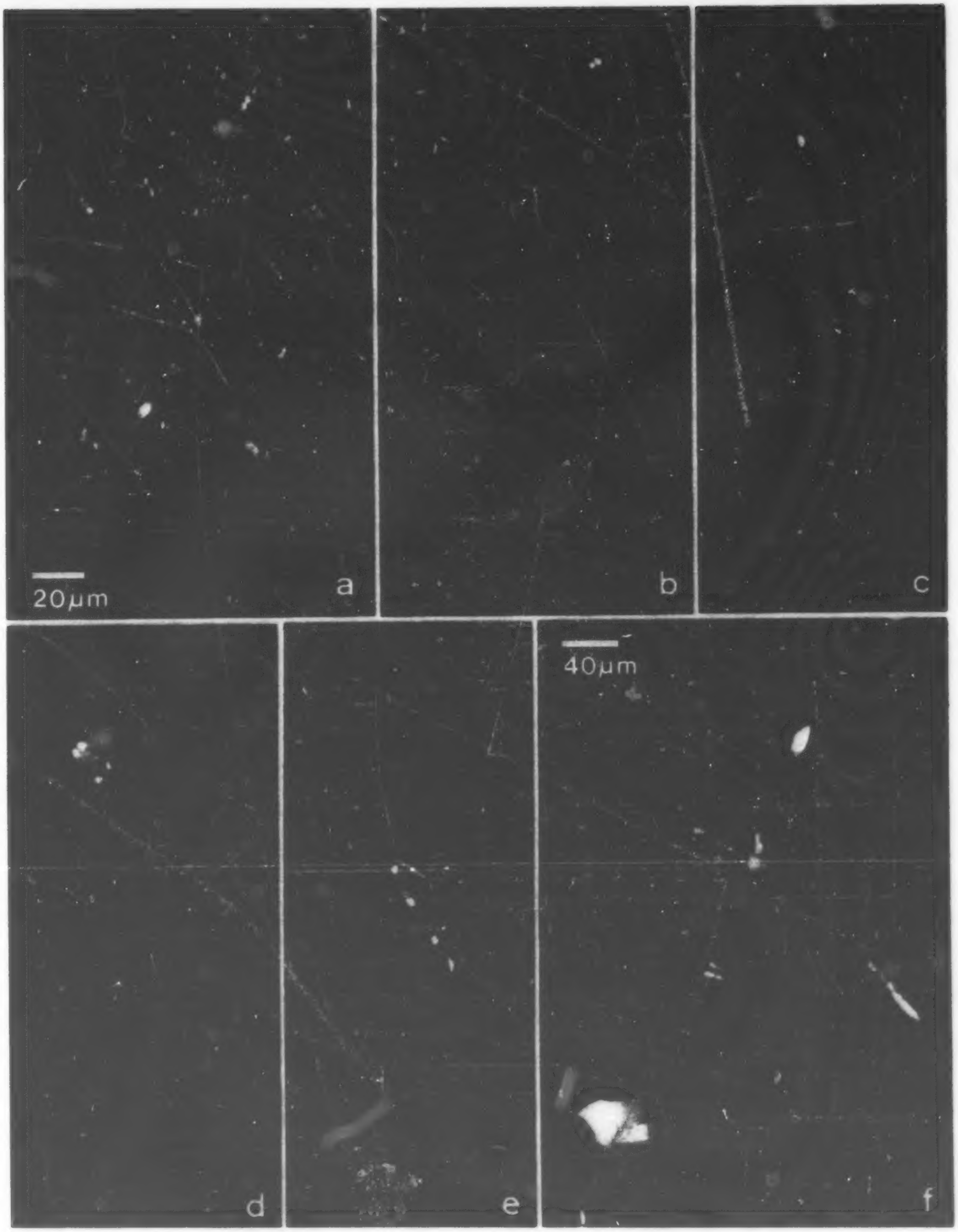


Figure 9. Fluorescence micrographs of nuclei in spores and germ tubes of *Endocronartium sahoanum*: a. two nuclei in spores; **b.** two nuclei in a young germ tube at about 6 h after germination; **c.** single nucleus in the tip of a germ tube at about 16 h.; **d.** four nuclei in a vesicle at the tip of a germ tube at about 20 h.; **e.** four nuclei in narrow pointed hyphae produced from the vesicle at about 24 h.; **f.** narrow pointed hyphae separated by a septum at about 48 h.

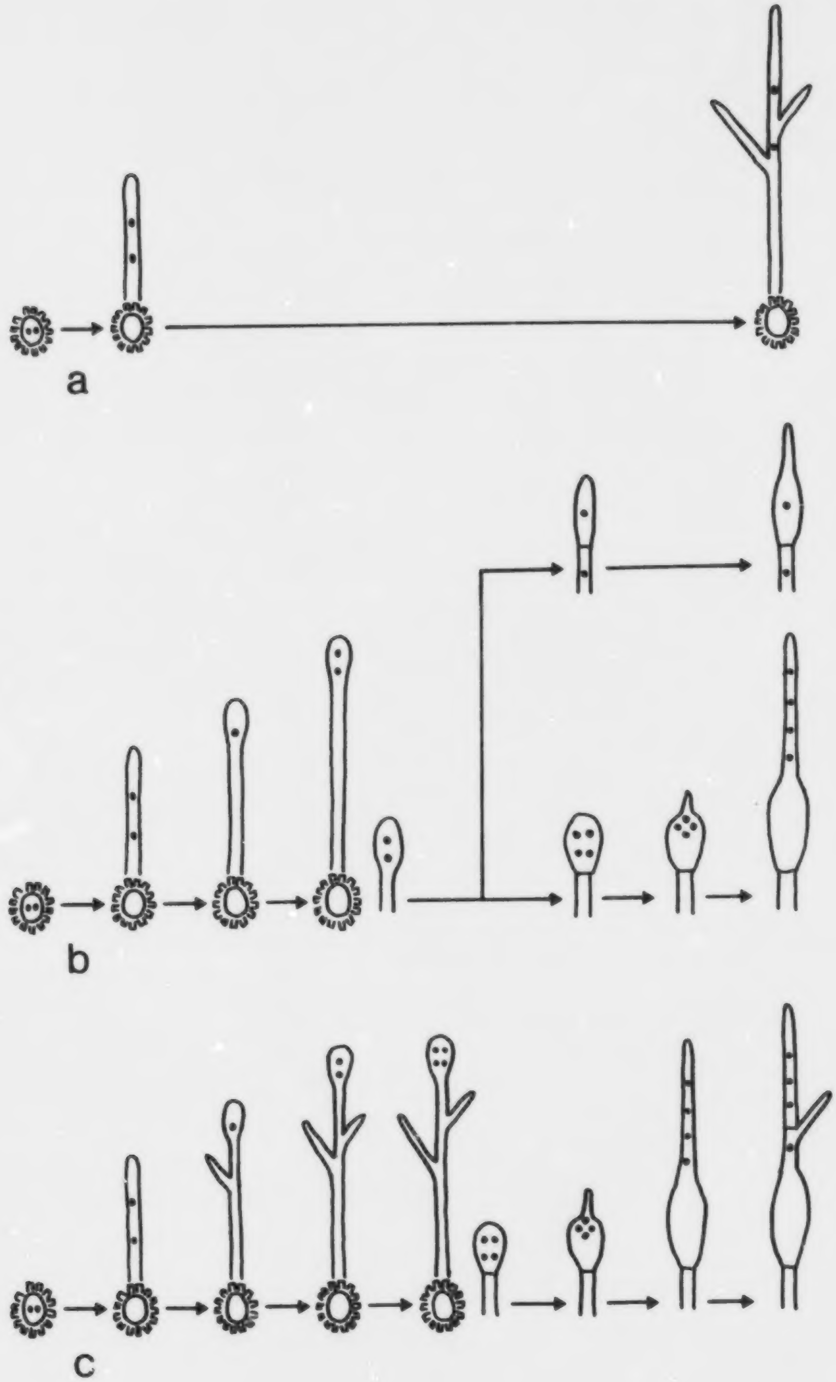
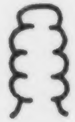
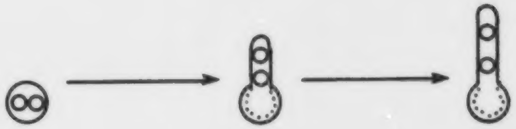
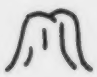
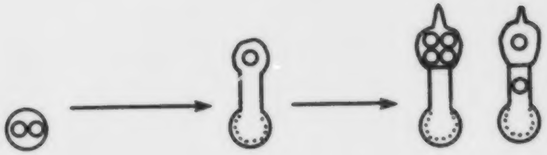
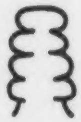
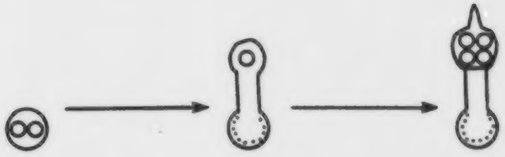


Figure 10. Nuclear behavior during spore germination: a. *Cronartium ribicola*; **b.** *Peridermium yamabense*; **c.** *Endocronartium sahoanum.*

Table 1. Comparative morphology of three rust species on five-needle pines in Japan

Species	Spore morphology		Nuclear behavior during spore germination
	Surface	Size	
Cronartium ribicola		20–32 × 13–23 (μm)	
Peridermium yamabense		23–40 × 16–31	
Endocronartium sahoanum		24–42 × 17–31	

P. pumila in this habitat. The habitat of *P. yamabense* is in the alpine zone in Hokkaido, where the flora is simple in terms of the plant species found, and only a few alpine shrubs (*Ledum* sp., etc.) grow around *P. pumila*. The habitat of *E. sahoanum* is located in the subalpine zone in Northern Honshu. Northern coniferous forest is well developed near the habitat of the rust, and *P. pumila* grows a little at the peaks and ridges of mountains, where *Sasa* spp. cover the ground.

From many geological studies, it is shown that it has generally become warmer in the past 30 000 years and the differences among the three types of vegetation have been brought about in the past 20 000 years, since the late Pleistocene (Miki 1938; Nasu 1972; Maekawa 1977; Yasuda 1980; Sohma and Tsuji 1988). With the transition of climatic and geographical conditions, the vegetation in Japan has changed (Yoshioka 1973; Yasuda 1980) (Fig. 11). During the coldest time of Würm Glaciation age, the climate of Japan and its vicinity was very cold and Japan was a part of continental Asia. Northern coniferous forest and tundra covered most of Japan at that time. During the late Würm Glaciation age, it got warmer, and northern coniferous forest and tundra have retreated to mountainous or northern areas of Japan. Since that time, it has become still warmer. At present, northern or boreal coniferous forest is distributed in mountainous areas in Central and Northern Honshu, and Hokkaido. Alpine tundra is distributed at high elevations in mountainous areas in Central Honshu and Hokkaido. Some plant species have disappeared, and some have adapted themselves to the new natural conditions over a long period. As a result, some new types of vegetation have formed in each area. It may be assumed that the stem rusts on *P. pumila* in Japan have also moved to high elevations or northern areas, and became isolated with *P. pumila*. The rusts have adapted themselves to the new environment and become separate species. Therefore, we consider the three species of stem rusts on *P. pumila* to have become separated as species since the late Pleistocene, because alpine tundra and northern or boreal coniferous forest, where *P. pumila* and some other five-needle pines occur, became geographically separated in Japan after the late Pleistocene.

We suggest that *P. yamabense*, which is morphologically different from *C. ribicola*, separated from an ancestor of *C. ribicola* or a heteromacrocyclic *Cronartium* species a long time ago. On the other hand, it seems that *E. sahoanum*, which is morphologically similar to *C. ribicola*, separated from *C. ribicola* in the period after the Pleistocene, because the habitats of *E. sahoanum* and *C. ribicola* have separated geographically in the postglaciation period. In conclusion, we propose the phylogeny of stem rusts on five-needle pines in Japan as illustrated in Figure 12. However, further research and studies of the rusts in Siberia, Kamchatka, Korea, Sakhalin, and Kuril Islands are required to clarify the phylogeny of the stem rusts in Japan.

ACKNOWLEDGMENTS

We sincerely thank Dr. Y. Hiratsuka, Northern Forestry Centre, Canada; Drs. S. Sato and Y. Yamaoka, Institute of Agriculture and Forestry, University of Tsukuba, Japan; and Dr. S. Kaneko, Tohoku Research Center, Forestry and Forest Products Research Institute, Japan, for their valuable suggestions for our study.

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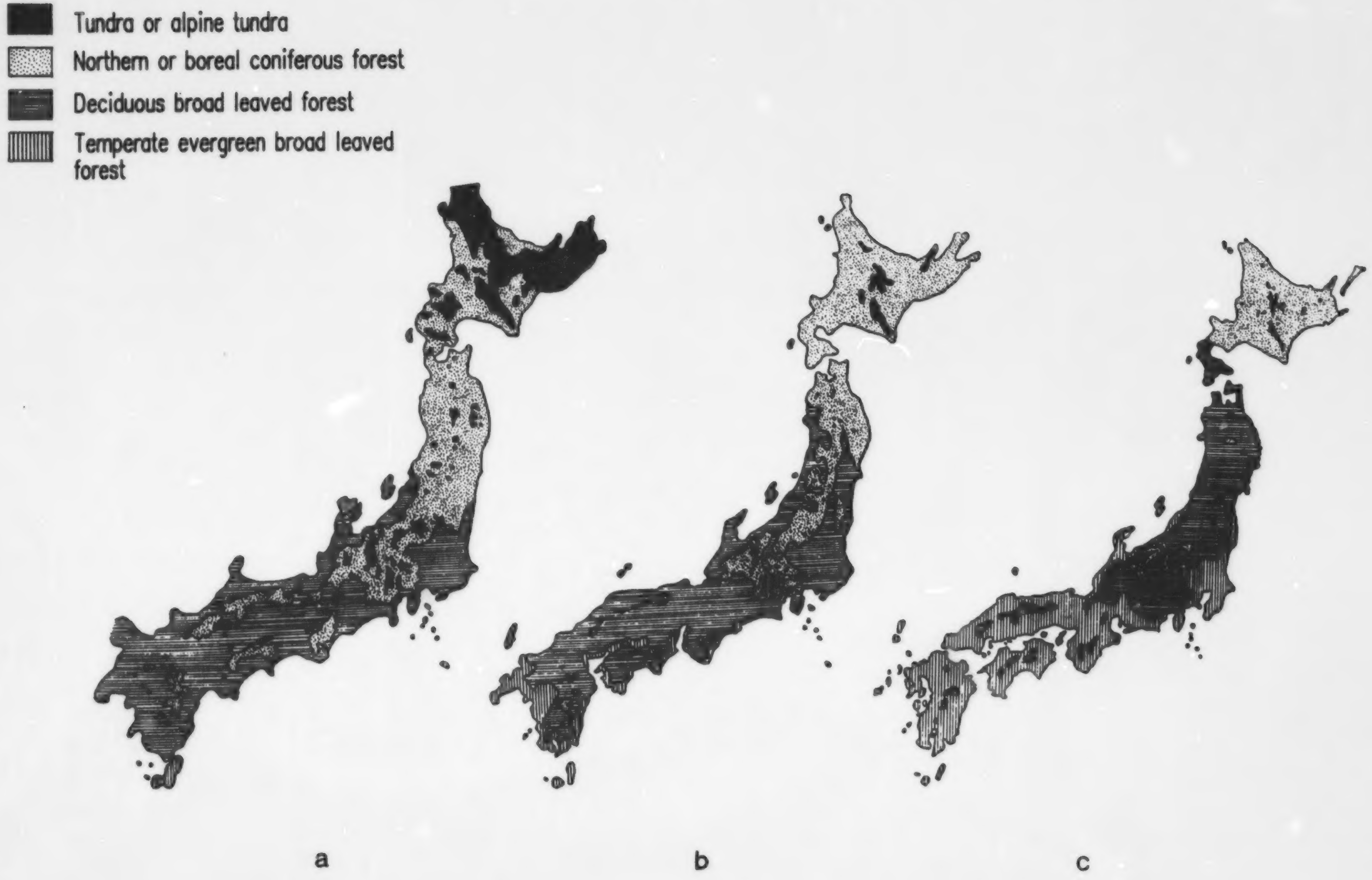
Tundra or alpine tundra
Northern or boreal coniferous forest
Deciduous broad leaved forest
Temperate evergreen broad leaved forest
a
b
c

Figure 11. **The transition of vegetation and topography in Japan, modified from Yasuda (1980) and Yoshioka (1973):** **a.** during the coldest time of Würm Glaciation (about 20 000 years B.P.); **b.** during the late Würm Glaciation (about 12 000 years B.P.); **c.** at present.

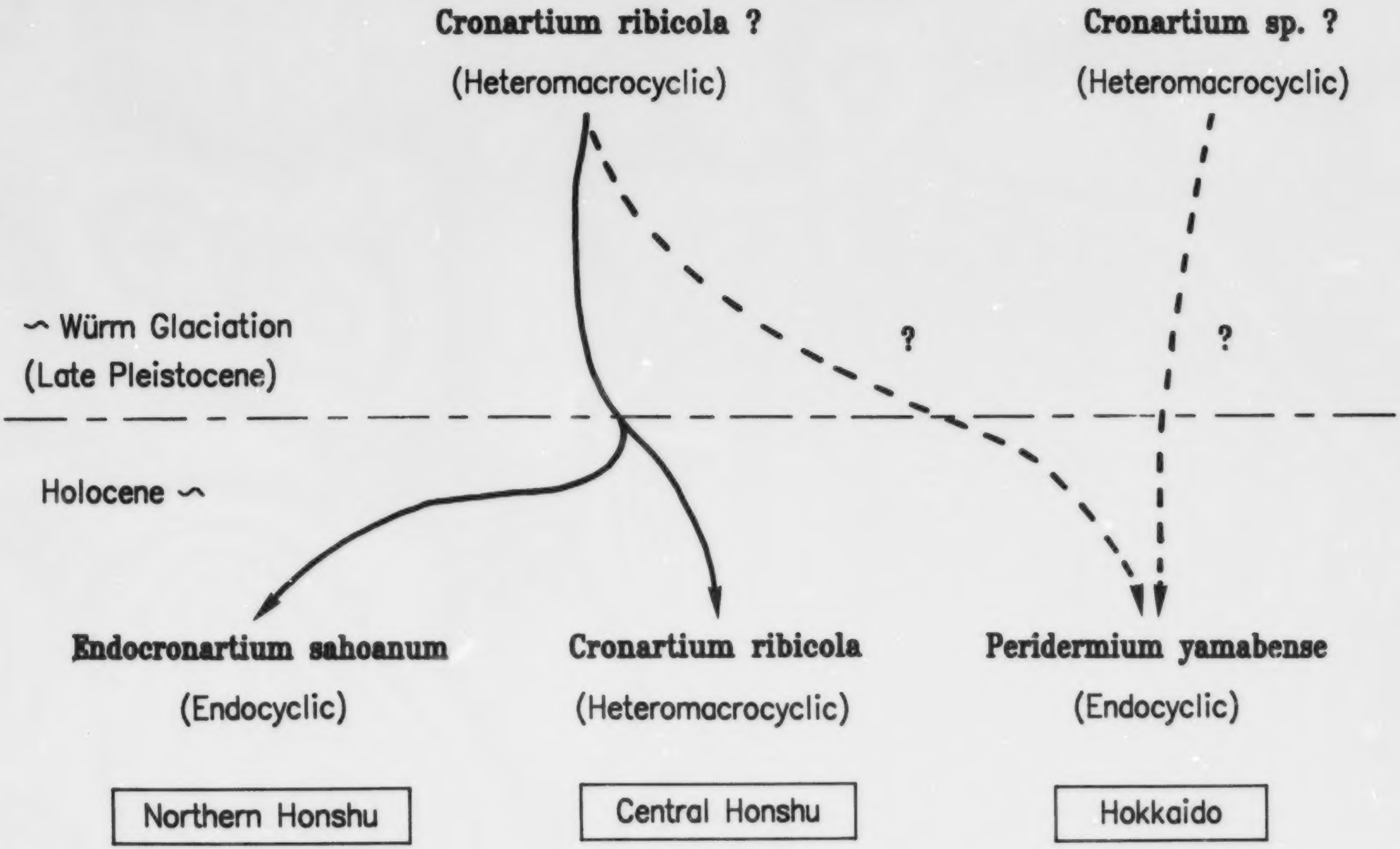
Cronartium ribicola ?
(Heteromacrocyclic)
Cronartium sp. ?
(Heteromacrocyclic)
?
?
~ Würm Glaciation
(Late Pleistocene)
Holocene ~
Endocronartium sahoanum
(Endocyclic)
Cronartium ribicola
(Heteromacrocyclic)
Peridermium yamabense
(Endocyclic)
Northern Honshu
Central Honshu
Hokkaido

Figure 12. The phylogeny of stem rusts on five-needle pines in Japan.

Hiratsuka, Y. 1986. Cytology of an autoecious soft pine blister rust (*Peridermium yamabense*) in Japan. Mycologia 78:637-640.

Hotta, M. 1974. [History and geography of plants.] Sanseido, Tokyo, Japan. P. 241-243. (In Japanese.)

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Miki, S. 1938. On the change of flora of Japan since the Upper Pliocene and the floral composition at the present. Jpn. J. Bot. 9:213-251.

Nasu, T. 1972. [Quaternary flora of Japan islands.] Biol. Sci. 24:1-10. (In Japanese.)

Saho, H. 1981. Notes on the Japanese rust fungi VII. *Peridermium yamabense* sp. nov.: a pine-to-pine stem rust of white pines. Trans. Mycol. Soc. Jpn. 22:27-36.

Sato, T.; Sato, S. 1985. Morphology of aecia of the rust fungi. Trans. Br. Mycol. Soc. 85:223-238.

Sohma, K.; Tsuji, S. 1988. [Quaternary flora of Japan.] Quat. Res. (Tokyo) 26:281-291. (In Japanese.)

Wicker, E.F.; Yokota, S. 1976. On the *Cronartium* stem rust(s) of five-needle pines in Japan. Ann. Phytopathol. Soc. Jpn. 42:187-191.

Yasuda, Y. 1980. [An introduction to environmental archaeology.] NHK Press, Tokyo, Japan. (In Japanese.)

Yokota, S.; Uozumi, T. 1976. New developments in white pine blister rust in Japan. Pages 330-343, Division II, *in* Proc. XVI IUFRO World Congress, June 20 to July 2, 1976, Oslo, Norway. Norw. IUFRO Congr. Commi., Norw. For. Res. Inst., Ås, Norway.

NUCLEAR CYCLE, TAXONOMY, AND NOMENCLATURE OF WESTERN GALL RUST

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ABSTRACT

In 1969, the endocyclic genus *Endocronartium* was established to include *E. harknessii* (J.P. Moore) Y. Hiratsuka (= *Peridermium harknessii* J.P. Moore) in North America and *E. pini* (Persoon) Leveille emend Klebahn (= *P. pini* (Persoon) Leveille emend Klebahn) in Europe, two autoecious pine stem rusts, but the justifications for establishing this genus have been questioned. Cytological events in spores and germ tubes of *E. harknessii* (western gall rust) were reexamined. Number of nuclei and relative DNA contents in various stages of spore germination, number and nature of septa and branches, and mode of initial host penetration suggested that the germlings of the two species function as metabasidia with nuclear fusion and meiosis, rather than as aeciospore germ tubes. It is concluded that the recognition of the endocyclic genus *Endocronartium* is justified and desirable.

INTRODUCTION

It is a well-established and accepted fact that the fungus causing the western gall rust *Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka (= *Peridermium harknessii* J.P. Moore) is autoecious (Hiratsuka et al. 1966; Nelson 1971; McKenzie 1942; Ouellette 1965; Wagener 1964; Zalasky and Riley 1963); the fungus does not have alternate hosts and is capable of infecting directly from pine to pine. Although there are a few reports of facultative autoecism or claims that this rust can act both as an autoecious and a heteroecious fungus at the same time (Anderson and French 1965; Fromme 1916; Meinecke 1920, 1929; Weir and Hubert 1917); it is now well accepted that this rust is autoecious.

In 1969, the genus *Endocronartium* was established to include *Peridermium harknessii* in North America and *Peridermium pini* in Europe, two autoecious pine stem rusts (Hiratsuka 1969), based on the morphology and cytology of germinating spores (Hiratsuka et al. 1966; Hiratsuka 1968). However, the reasons for establishing this genus and nomenclatural interpretations have been questioned (Laundon 1976). Recently, Epstein and Buurlage (1988) disagreed with the interpretation of observations and with the taxonomic decisions of Hiratsuka (1969) and suggested that the fungus should be called by the anamorphic name *Peridermium harknessii*. The main reason for their justification was that they did not find evidence of nuclear fusion and meiosis during the spore germination. Their interpretation of the nuclear cycle of the fungus is shown in Figure 1. They concluded that no nuclear fusion or meiosis occurred in the spores or germ-tubes and that only mitosis occurred in the germ tubes. They also failed to recognize the common presence of second and third septa in the germ tubes. Their observations and nomenclatural conclusions are therefore subject to different interpretations. In this paper, additional evidence to support the endocyclic life cycle of *E. harknessii* is given.

The problem should be divided into three separate aspects for logical evaluation and making taxonomical and nomenclatural decisions. It is neccessary 1) to observe and understand what is happening morphologically and cytologically before and after spore germination; 2) to consider the interpretation of

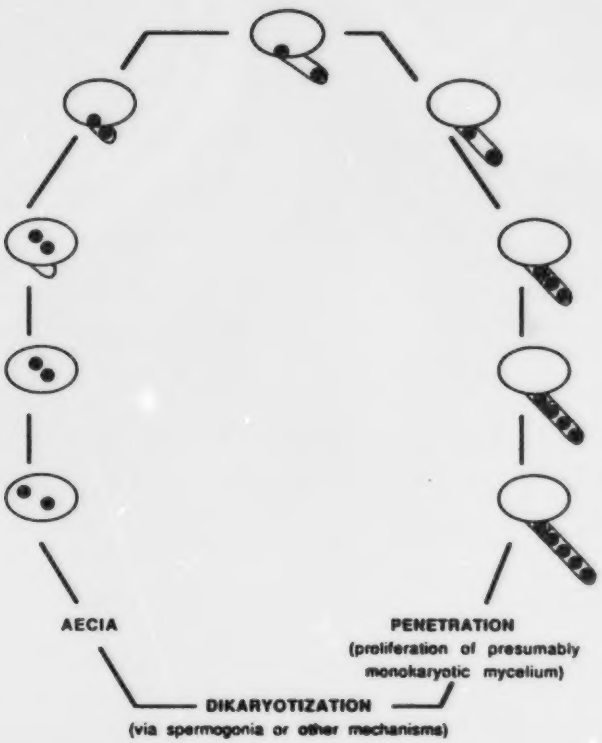

AECIA
PENETRATION
(proliferation of presumably
monokaryotic mycelium)
DIKARYOTIZATION
(via spermogonia or other mechanisms)

Figure 1. Nuclear cycle of western gall rust proposed by Epstein and Buurlage (1988).

recognized facts, i.e., to understand and interpret the nature of the germ tubes; and 3) finally, based on 1) and 2) above, to make taxonomical and nomenclatural decisions.

MORPHOLOGY, CYTOLOGY, AND NATURE OF SPORES AND GERM-TUBES

In view of the method used by Epstein and Buurlage (1988), we reexamined fresh germinating spores using epifluorescent microscopy with DAPI staining, as well as spores and germ tubes stained earlier with HCl-Giemsa and Iron-Haematoxylin. We used a Zeiss Photometer to measure relative amounts of DNA in DAPI-stained nuclei at critical stages of development. Our work is based mainly on forms of western gall rust existing in western Canada but also includes earlier observations of samples collected from Colfax, California, the type locality of the fungus.

Aeciospore germ tubes of a heteroecious species such as *Cronartium coleosporioides* are an unseptated and indeterminate type with the two nuclei migrating into germ tubes. No nuclear fusion or division occurs during germination. Two (or occasionally three) nuclei in the spores simply migrate into the germ tubes, thus no nuclear fusion or division figures are observable in the germ tubes. Upon infection on the alternate host plant, they establish dikaryotic mycelium; therefore, no change in nuclear status occurs in the spore during germination and after infection. Germ tubes of western gall rust are usually septated into three, four, or five segments and the growth is determinate. Germ tubes often have side branches mostly from the first cell of the germ tube. One germ tube produces as many as three branches. The side branches as well as tips of the germ tubes are capable of causing infection (Hopkin et al. 1988). Epstein and Buurlage (1988) found no evidence that side branches were involved in host penetration, but our evidence clearly indicates that they are functional. SEM pictures reported in Hopkin et al. (1988) support this statement (Figs. 2, 3). Observations further indicated that germ tubes on the susceptible host plant surface tend to be shorter than those on thin water agar or on a film of water.

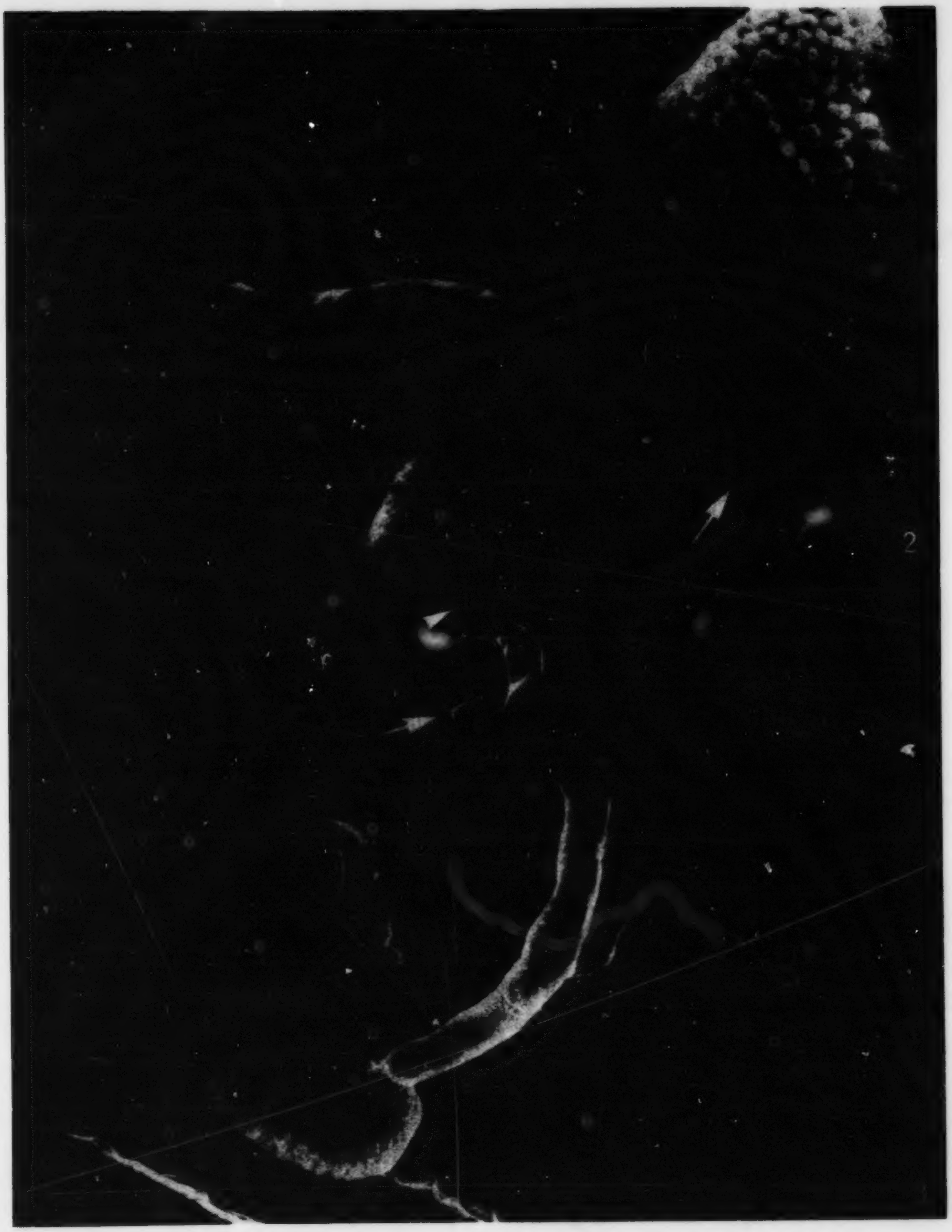
Nuclear events in the germ tubes of western gall rust are also very different from heteroecious species (Hiratsuka et al. 1966). Hyphae in the pine gall tissue are monokaryotic and likely haploid. Most young spores possess two nuclei, which means dikaryotization takes place at the base of the sorus. This is the same as in aeciospores of heteroecious species. However, upon germination, active nuclear divisions occur in the germ tubes which divide eventually into two to five segments by septa. Each segment of a septated germ tube usually has one nucleus. Dikaryotization and de-dikaryotization is clearly taking place here (Fig. 4). This is clearly different from imperfect states of rusts in which dikaryotic spores, either aeciospores or urediniospores, germinate and two nuclei migrate into germ tubes without nuclear fusion or divisions.

Is karyogamy or nuclear fusion and meiosis involved in this process of de-dikaryotization? In my opinion, there is good evidence of nuclear fusion and meiosis during germination.

Variable percentages of spores with one nucleus exist just before germination. The number of spores with one nucleus were observed to increase during the first 2 h of incubation. Figure 5 is an example of one observation. Observations of single nuclei were classified as fuzzy or dense because they may represent different kinds of nuclei. The fuzzy-type nuclei are probably fused or fusing diploid nuclei, and the dense type are likely two haploid nuclei that look like one because of overlap. Besides numbers, single nuclei and dikaryotic nuclei differed in morphology in both DAPI-stained spores and Giemsa- and Haematoxylin stained material. For this purpose, well-stained slides with Giemsa were superior to those stained with DAPI. Dikaryon nuclei are evident in young spores (Fig. 6). Many monokaryotic nuclei have somewhat diffused chromatin; thus the nuclei look large and fuzzy (Figs. 7, 8). In fact many spores have two chromatin masses close together and often look like one, as also suggested by Epstein and Buurlage (1988), but presumably many of them are in the process of nuclear fusion. Such a phenomenon is very rare in truly dikaryotic spores and germ tubes of heteroecious species.

Germination and nuclear division occur within 2-4 h of incubation. Various nuclear migration and division figures are observed between 4 and 6 h in young germ tubes (Figs. 9-14). Usually nuclei seem to come out of the spores in strings of chromatin bodies (Figs. 9, 10). In this stage it is difficult to say if there are one or two nuclei. Figures 7 and 8 show diffused nuclei of what are probably premeiotic diploid nuclei. No such nuclei have been observed in germ tubes of heteroecious species.

Many young germ tubes had one nucleus. The single nucleus divides into two (Figs. 11, 12, 13), then divides again into four (Fig. 14). But we also observed one nucleus still in the spore and one in the young germ tube or one already in the germ tube and one emerging from the spore. Also, nuclear divisions producing four nuclei are often not synchronous. These facts have been pointed out by Epstein and Buurlage (1988) as evidence that meiosis is not happening during the process. However, the second division of meiosis is not always synchronous, and the first division could have happened in the spore before germination. Time lapse photography shows that nuclei and other cytoplasmic contents are actively moving in the first few hours of germination, and cell contents often go back and forth, including back



Figures 2, 3. Germ tubes of ***Endocronartium harknessii*** showing host penetration by side branches (arrows), as well as the tips of the germ tubes (arrowheads). (Fig. 2, × 1100; Fig. 3, × 500)

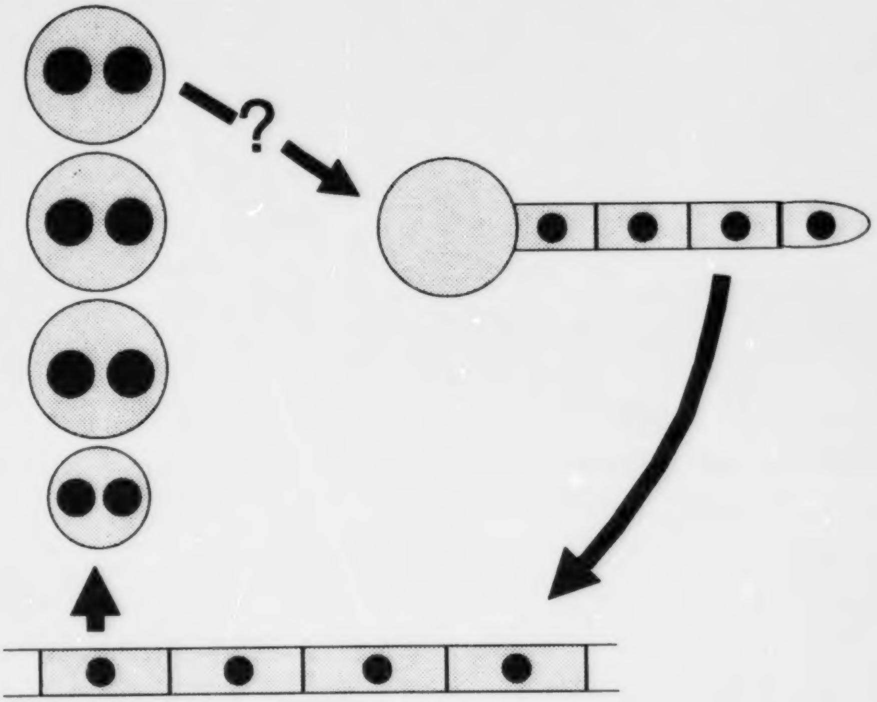
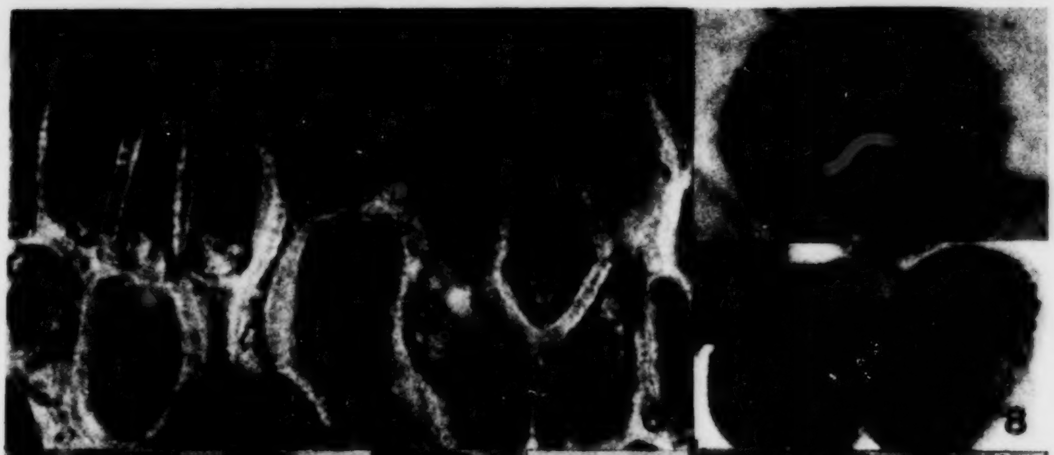
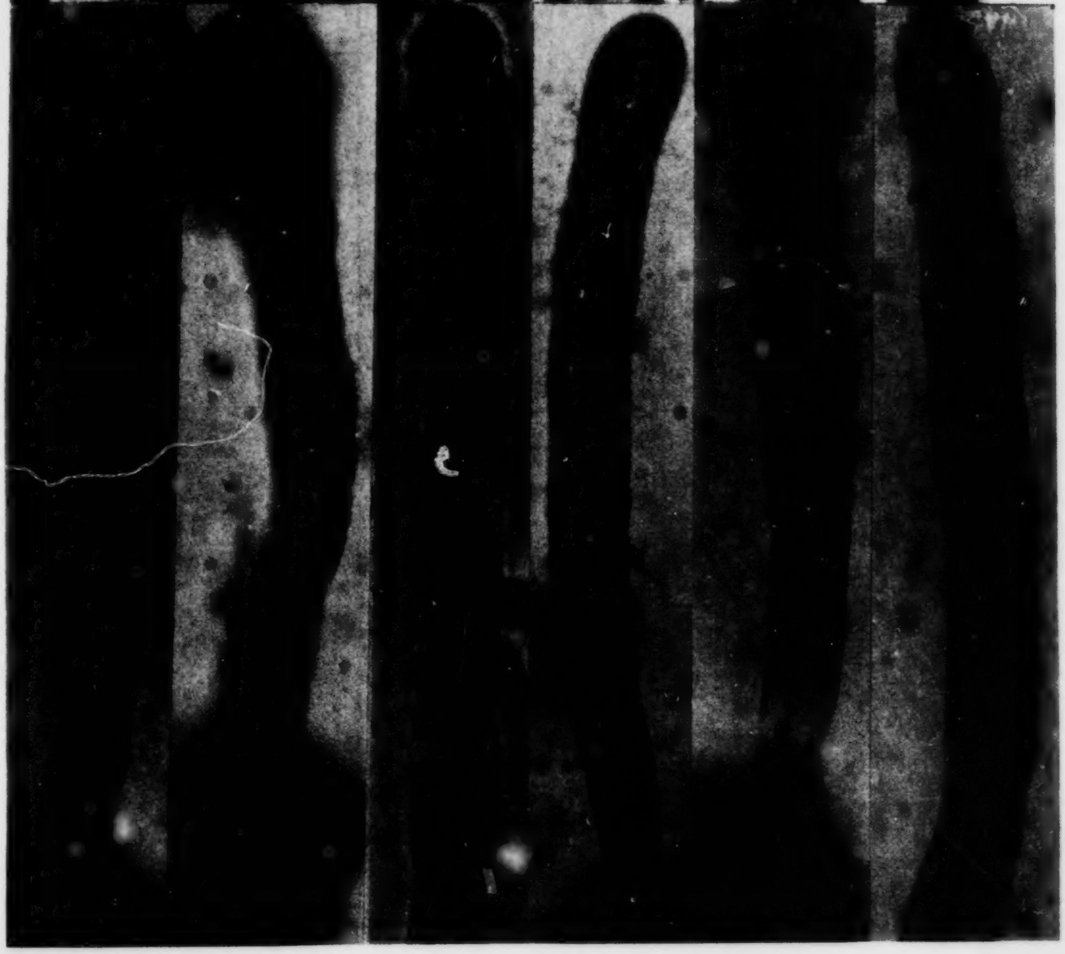


Figure 4. Alternation of nuclear states in *Endocronartium harknessii.*

Incubation time, h	Number of nuclei			
	1		2	3
	Fuzzy	Dense		
0	13.0	10.3	72.9	3.9
1	38.6	2.8	57.4	1.2
2	54.7	5.3	39.1	0.9

Figure 5. Percentage of *Endocronartium harknessii* spores with 1, 2, or 3 nuclei after different incubation times.





Figures 6-8. **Nuclei in *Endocronartium harknessii* spores.** **6.** Binucleate condition of young spores in a sorus. Iron-Haematoxylin staining (× 800). **7, 8.** Nuclei of *Endocronartium harknessii* spores just before germination. Dense dikaryotic nuclei (arrows) and diffused diploid nuclei (arrowheads). HCl-Giemsa staining (× 900).

Figures 9-14. **Various nuclear events in the germ tubes of *Endocronartium harknessii* after 6 h of incubation.** HCl-Giemsa staining (× 1000).

into spores. Germ tube elongation, septations, and nuclear divisions stop about 12 h after incubation. Further nuclear divisions may occur in the extended tips and branches.

With a Zeiss Photometer, measurements were made of the relative amount of DNA in two kinds of nuclei in DAPI-stained germ tubes (Fig. 15): single nuclei after 6 h of incubation which were assumed to have just emerged into the germ tubes; then nuclei in older germ tubes, after 16 h of incubation, which were predicted to be mostly haploid. In the first group of nuclei, peaks appeared around 100 and again about 200. In the second group, a peak appeared between 40 and 60 and tapered toward 100 to 120. This means that nuclei just emerged from spores have more DNA than nuclei of older germ tubes. If nuclei in the 40-60 range are haploid, nuclei having values of about 100-120 are diploid and nuclei with 200-220 values are double that of the diploid.

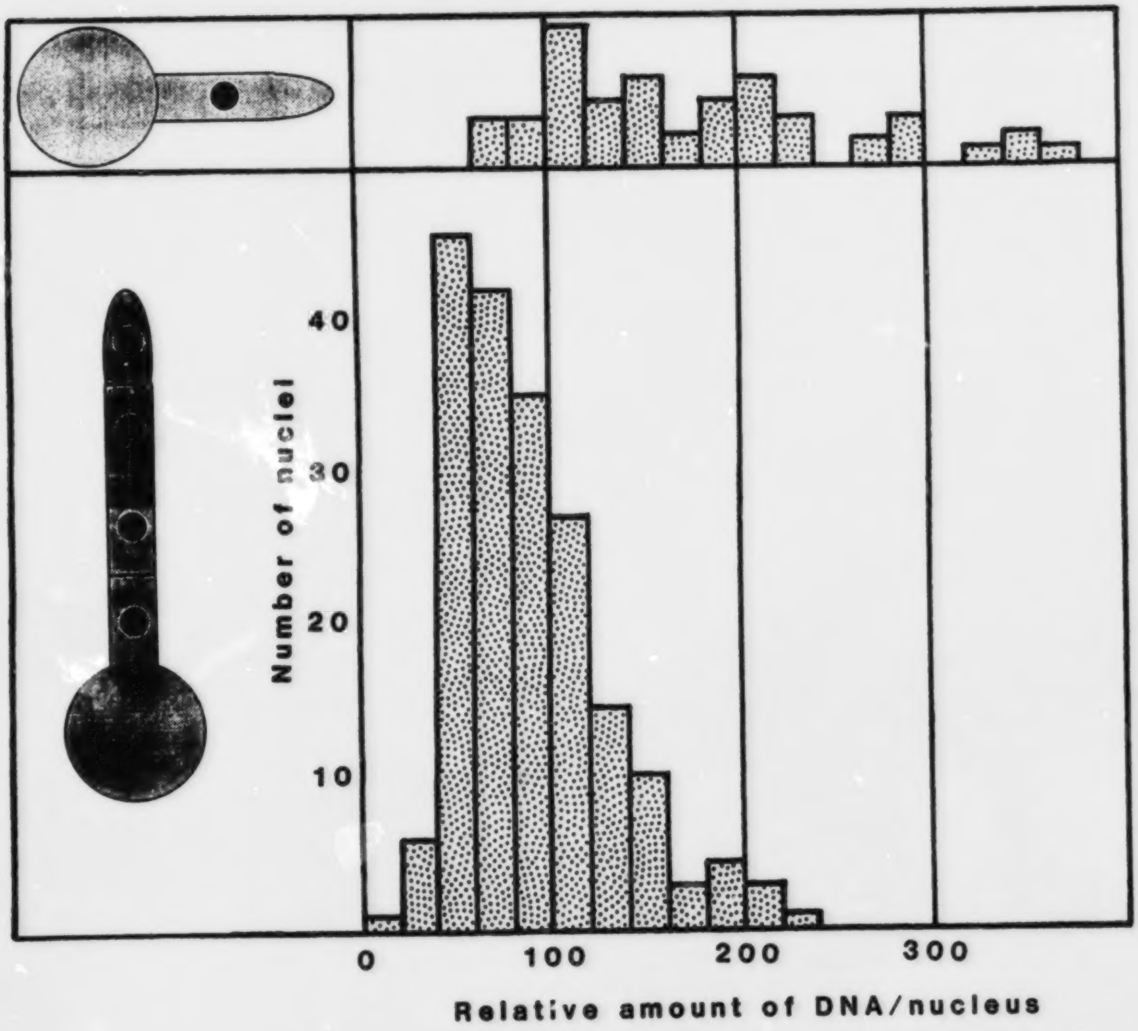


Figure 15. **Relative amounts of DNA in germ tubes of *Endocronartium harknessii* incubated for 4 (top) and 12 h (bottom).**

Although not conclusive, these data strongly suggest that meiotic divisions were involved in the process. If only mitotic divisions were involved, values for the two different stages should not differ. The final conclusive evidence of nuclear fusion and meiosis will be to observe synaptonemal complexes during the prophase I nuclei by transmission electron microscopy.

INTERPRETATION OF OBSERVED FACTS

The next point of consideration is the interpretation of the observed facts of spores and germ tubes of western gall rust and to decide the nature of the germ tubes and spore state. Three interpretations are possible: 1) typical aecia, 2) repeating aecia (uredinoid aecia), or 3) endocyclic telia or peridermioid telia. The first two possibilities would suggest that this fungus has only an anamorph, in which case the fungus is known only as an imperfect state; but the third interpretation would suggest this fungus to have teleomorph, thus is a perfect fungus.

The most desirable interpretation is to consider septated germ tubes of the western gall rust as homologous to basidia (metabasidia) rather than regular germ tubes of aeciospores. Even if nuclear fusion and meiosis do not occur regularly during de-dikaryotization, it is more difficult to interpret the germ tubes of western gall rust as regular aeciospore germ tubes in which no nuclear fusion or division should occur. Jackson (1935) described six different types of monokaryotization in microcyclic and endocyclic rusts with or without nuclear fusion and typical meiosis. Hiratsuka and Sato (1982) summarized various types of nuclear events in rust fungi before and after metabasidia formation, including types not involving nuclear fusion and meiosis. Therefore, based on the evidence, this fungus should be considered as perfect fungus having an endocyclic life cycle.

TAXONOMY AND NOMENCLATURE

Based on the observations and interpretations of the nature of germ tubes as homologous to basidia (metabasidia), the taxonomy and nomenclature of the fungus should be reviewed.

If we agree that the fungus should be treated as a perfect fungus having an endocyclic life cycle, we cannot keep this fungus in the imperfect genus *Peridermium*.

When Hiratsuka (1969) established the genus *Endocronartium* to include western gall rust (*E. harknessii*) and another form from Europe (*E. pini*), he presented three possible options for the nomenclature of the fungus as follows: 1) include the species in the parental genus *Cronartium*, 2) recognize the two fungi as belonging to one of the existing endocyclic genera, such as *Endophyllum*, *Gymnoconia* (*Kunkelia*), or *Monosporidium*, or 3) establish a new genus. He gave reasons for choosing to establish a new endocyclic genus.

Since *Peridermium pini* is the type species of the genus *Peridermium*, it was considered difficult to transfer the type species of a genus to another genus (Laundon 1976). If the type of *P. pini* can be proven to be the pine-to-pine race, the generic name *Peridermium* needs to be used for the endocyclic species and the concept of the genus *Peridermium* as now applied will be changed. However, Hiratsuka (1969) pointed out that the species has been divided into two different species (*P. pini*, pine-to-pine form; and *P. cornui*, host-alternating form) and descriptions were emended by Klebahn (1890), thus the original *P. pini* as described by Link (1816) is *nomen ambiguus* (an ambiguous name which cannot be applied for a specific organism). To clarify the situation and to avoid unnecessary changes in the concept of *Peridermium*, and to conserve the *Peridermium* as an imperfect genus, a new type *P. elatinum* was proposed (Hiratsuka 1974) and was accepted at the XIII International Botanical Congress, Sydney, Australia 1981.

CONCLUSIONS

Germ tubes of western gall rust should be considered homologous to basidia (metabasidia) rather than germ tubes of an anamorphic fungus. This fungus should be recognized as a perfect fungus having endocyclic life cycle. The name *Endocronartium harknessii* is the most appropriate name of the pathogen of the western gall rust.

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ANALYSES OF PROTEINS OF WESTERN WHITE PINE (*PINUS MONTICOLA* DOUGL.) NEEDLES

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INTRODUCTION

Today, I will talk on the analyses of proteins of western white pine needles. Western white pine is of significant commercial value and can be grown in areas where other species are susceptible to root rot disease. Therefore, there is a renewed interest in planting western white pine in British Columbia (Meagher and Hunt 1985). However, one of the problems with western white pine is the blister rust caused by *Cronartium ribicola* J.C. Fisch ex Rabh. The approach taken is to breed blister rust resistant pine trees. The selection criteria of resistant seedling is based on the analyses of morphological characteristics, for example, frequency of needle spots, their color, shedding of needles, short shoot reaction, bark reaction, or no reaction at all which are seen following infection with rust fungus. Whether the resistant traits of the trees are indeed associated with these characteristics could truly be evaluated after field tests which may take 10-15 years. The process of selection is very time-consuming and costly. A quicker and perhaps cheaper method is based on a biochemical marker such as a resistant gene or its product, i.e., proteins need to be developed. To develop this type of methodology, it is imperative that we understand the biochemical mechanism underlying the disease process. Most important, an elucidation of this mechanism will lead to the identification of desired biochemical markers. With the advent of biotechnology, it is envisaged that the availability of these markers would also facilitate the genetic engineering of resistant trees.

Let us examine some of the approaches one might take towards the understanding of the disease process of the white pine blister rust. Having spent a great deal of time in medical research, the immediate question comes to my mind: how does the basidiospore of the rust fungus attach itself onto the needle surface? Is there any recognition molecule(s), such as receptors, on the needle surface through which the first phase of plant-fungal interaction takes place? The nature of interaction may well dictate the type of signal to be received by the host's defense system. In support of the receptor concept, one can perhaps cite the recent work demonstrating that the attachment of rice blast fungus could specifically be blocked by concanavalin A (Hamer et al. 1988). In another study (Epstein et al. 1987), the presence of adhesion proteins of bean rust fungus have been documented. Analyses of the plant-fungal interaction would necessitate the identification of proteins of both needle and basidiospores. Once identified, appropriate monospecific polyclonal or monoclonal antibodies can be produced against individual components. These antibodies can then be utilized in a suitable experimental design to block the interaction between the pathogen and its host and establish how these gene products are integrated into processes that are unique to each system.

The immediate need of the breeders of blister rust resistant white pine is the development of biochemical methods which would select for the desired trait, for example, resistance. There are two approaches to this: 1) to establish a protein data base (based on 2-D gel analyses of needle, seed and pollen proteins) of a large number of both resistant and susceptible trees present in the natural stand and, with the availability of sophisticated laser gel scanner and computer software, one may be able to establish protein profile characteristics of resistant and susceptible trees and thus identify proteins that are associated with the resistant trait; 2) to directly infect young seedlings with the rust fungus and analyze proteins by 2-D gel followed by gel scanning and necessary computation. A major disadvantage in the second approach is the detection limit of the techniques which, in time, would hopefully evolve to more sensitive methods. Another potential problem is the distinction between pathogen-induced host proteins and the pathogen proteins themselves. However, with the availability of appropriate anti-fungal antibodies, this problem can be resolved.

RESULTS AND DISCUSSION

With these objectives in mind, the first phase of the work in my laboratory is to develop extraction procedures for the needle proteins that are amenable to the electrophoretic analyses. The initial extraction procedure is shown in Fig. 1. Needles were collected by Dr. Richard Hunt of our center from pine trees in Lens Creek area of Vancouver Island last summer, and were kindly provided for this study. Needles were lyophilized, defatted and then dried. They were ground to powder and extracted with buffer. You will note that the extraction buffer contains sulphite mixture which was added to prevent the oxidation of phenols. These phenolic products are known to interfere with the extraction of proteins. PMSF, a protease inhibitor, was added to prevent the degradation of proteins by plant proteolytic enzymes that might be activated during extraction. The extract was filtered and centrifuged. The resulting supernatant was dialysed and lyophilized. The immediate problem was encountered with the protein determination of the lyophilized extract as illustrated in Table 1.

The lyophilized extract was redissolved in water at a concentration of 0.5 mg/mL. Protein content was determined by both Lowry and Bradford's dye binding assay. I was surprised to see a high protein value obtained with Lowry and, on precipation with 10% TCA, the protein value was drastically reduced. Dye binding method gave a different set of values. At this point, the extract, at varying amounts, was analyzed by SDS polyacrylamide gel electrophoresis and no protein band could be detected on the gel. Moreover, when higher concentration of the extract was used, the distortion of standard protein marker was observed, indicating the presence of materials in the extract that interferes with the electrophoretic process. Having failed to detect any reasonable protein bands on the gel, we asked ourselves whether we indeed extracted any proteins from the needles. To answer this, this sample was hydrolysed and the amino acid content of the hydrolysate was analyzed by automatic amino acid analyzer. As you can see, the protein content as estimated by this method was extremely low, which explains the lack of protein bands on the gel. None of these methods gave a true estimate of the protein content of the extract. Therefore, one has to be cautious as to the interpretation of results based on equal protein content of needle extract.

Table 2 shows that the substances that interfered with the protein determination also absorbed at 280 nm. Thus, the absorbency of the extract at 280 nm/mg of lyophilized weight was compared with absorbency per milligram of Lowry protein and there was a good correlation. To confirm the absorption characteristics of the interfering substances, needle extract was precipitated with ammonium sulphate at 75% saturation. The supernatant was dialysed and lyophilized and redissolved in water. Although the protein value of the supernatant as determined by Lowry was still high, no protein could be detected by dye binding assay. This absorption spectrum as shown in Fig. 2 clearly indicates that the interfering substances had absorption maximum at 275 nm. One could possibly establish the identity of this interfering substance by separating these compounds by PHLC. Since I did not want to get sidetracked at this stage, I adopted another extraction procedure as schematically shown in Fig. 3.

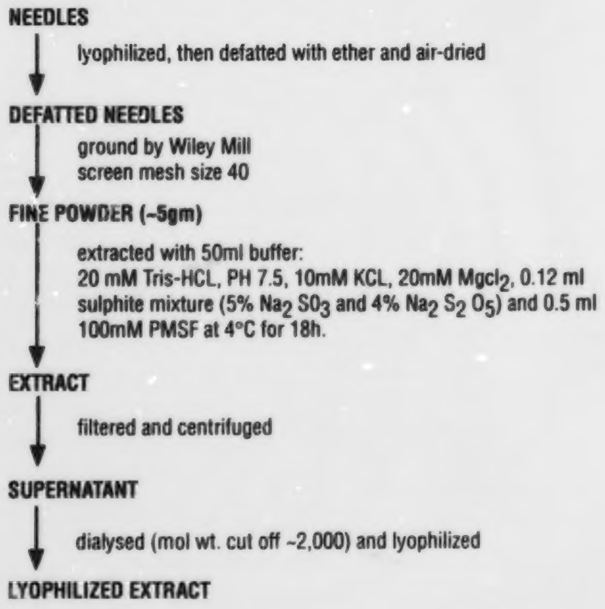
NEEDLES
lyophilized, then defatted with ether and air-dried
DEFATTED NEEDLES
ground by Wiley Mill
screen mesh size 40
FINE POWDER (~5gm)
extracted with 50ml buffer:
20 mM Tris-HCL, PH 7.5, 10mM KCL, 20mM Mgcl$_2$, 0.12 ml
sulphite mixture (5% Na_2SO_3 and 4% $Na_2S_2O_5$) and 0.5 ml
100mM PMSF at 4°C for 18h.
EXTRACT
filtered and centrifuged
SUPERNATANT
dialysed (mol wt. cut off ~2,000) and lyophilized
LYOPHILIZED EXTRACT

Figure 1. Flow sheet of the extraction of western white pine needle proteins (Method I).

Table 1. Problem associated with the determination of western white pine (WWP) needle proteins

			Protein determination by:			
WWP tag no.	Clone no.	Concentration (mg/mL)	Lowry (% protein)	Lowry after TCA pptn (% protein)	Dye binding (Bio Rad) (% protein)	Amino acid analysis (% protein)
3790	G-27	0.5	264	5.0	19	0.22
3790[a]	G-27	0.5	280	5.8	28.4	0.23
3795	6149	0.5	236	5.8	24	0.34
3807	B643	0.5	200	4.8	19	0.46
3866	G-161	0.5	232	4.8	15	0.34

[a] Needles were not defatted.

Table 2. Absorption of interfering substance(s) at 280 nm

WWP	A_{280}/mg lyophilized weight	A_{280}/mg Lowry protein
3790	2.54	2.20
3790[a]	2.55	2.40
3795	2.54	2.36
3807	2.30	2.52
3866	2.55	2.43

[a] Needles were not defatted.

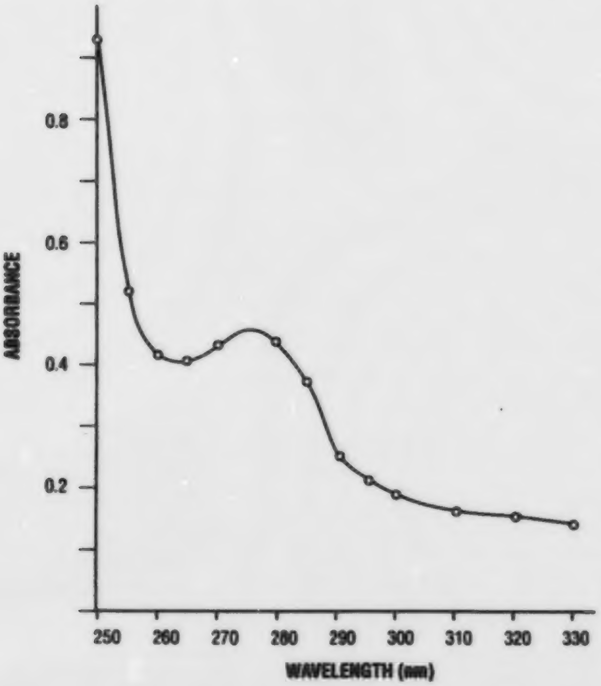

Figure 2. Absorption spectrum of interfering substance(s). Concentration: 0.096 mg/mL.

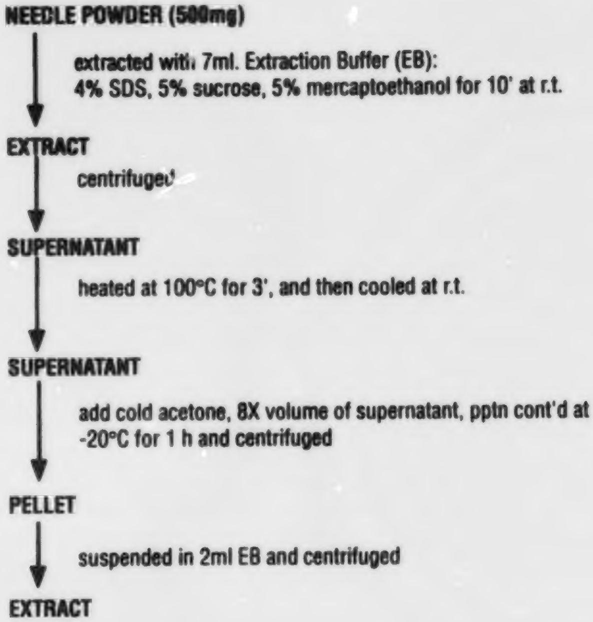
NEEDLE POWDER (500mg)
extracted with 7ml. Extraction Buffer (EB):
4% SDS, 5% sucrose, 5% mercaptoethanol for 10' at r.t.
EXTRACT
centrifuged
SUPERNATANT
heated at 100°C for 3', and then cooled at r.t.
SUPERNATANT
add cold acetone, 8X volume of supernatant, pptn cont'd at
-20°C for 1 h and centrifuged
PELLET
suspended in 2ml EB and centrifuged
EXTRACT

Figure 3. Flow sheet of the extraction of western white pine needle proteins (Method II).

In this procedure, an ionic detergent (SDS) and a reducing agent (mercaptoethanol) were added to the extraction buffer. The procedure also involves boiling the extract and concentration of proteins by adding acetone. The advantage of this method is that it should inactivate: 1) the enzyme polyphenol oxidase, thus preventing the oxidation of phenolics and their subsequent binding to proteins, and 2) a wide variety of plant proteolytic enzymes. In addition, the method allows for the solubilization of membrane proteins and thus the examination of more gene products, i.e., proteins. The disadvantage of the method is that any proteins isolated cannot be examined for a given biological activity. Another disadvantage is that both SDS and mercaptoethanol interfere with various protein determination methods. For comparison of various samples, therefore, the amount of starting material, volume of extraction buffer during extraction and reconstitution were kept constant for each sample.

The electrophoresis was initially carried out in Pharmacia Phast automated electrophoresis equipment and the gel was stained with silver. The advantage of the system is that a total of 24 samples and as little as 0.3 μL volume containing only a nanogram quantity of proteins can be analyzed in a half a day. Having established the suitable extraction procedure and the electrophoretic conditions, needle proteins obtained from four trees were compared as shown in Fig. 4.

The SDS-PAGE shown here was, however, carried out in a standard BioRad vertical slab gel apparatus and a volume of 15 μL of sample was applied. The gel was stained with coomassie blue. Four trees were examined: 3790 and 3795 are susceptible, and 3807 and 3866 are resistant type. In the second part of the slide, proteins were extracted in the presence of insoluble polyvinyl pyrrolidone, which is known to facilitate the extraction of proteins in the presence of phenolics. As you can see, there is no advantage to using PVP in this extraction procedure that we have adopted. You will note that there is an

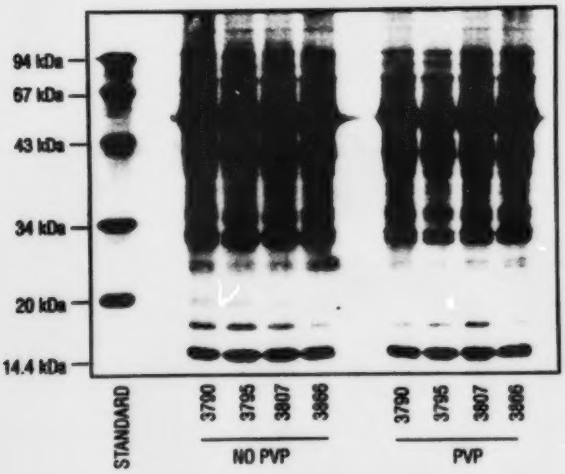

Figure 4. SDS-PAGE analysis of western white pine needle proteins as extracted by Method II (cf. Fig. 3). The electrophoresis was carried out on 12% gel, 15 µL volume of sample was applied, gel was stained with coomassie blue. The first lane represents the molecular weight markers.

abundance of 55 kDa proteins in all four trees, and it may represent large subunits of the enzyme ribulose biophosphate carboxylase-oxygenase (Rubisco). A total of 26 proteins well resolved could be detected in these trees. The important point here is that no obvious qualitative differences could be observed among these four trees as far as the major proteins were concerned. However, in the absence of gel scanner, the quantitative differences among these components could not be evaluated at this point in time.

While determining the protein content of the extract by amino acid analysis, it was found that all four trees had high glycine-rich proteins (Table 3). This finding, I believe, is significant for two reasons: 1) there are two types of cell wall proteins (proline-rich extensin and glycine-rich unidentified protein) that have been implicated in plant defense mechanisms and, furthermore, where there is no extensin present in the plant it seems to have glycine-rich proteins (for review see Cassab and Varner 1988); 2) the enzyme chitinase is also a glycine rich protein (Broekaert et al. 1988). It cleaves chitin, a polysaccharide component of fungal cell wall, and has been shown to inactivate the fungi in several host-pathogen interactions. There is some histological evidence (Martin 1967) that chitin is present in the fungal cell wall of the blister rust fungus, *Cronartium ribicola*. I am now actively pursuing the isolation of glycine-rich protein in attempts to study its regulation in white pine in response to infection with the rust fungus.

CONCLUSION

The inaccuracy of the available methods for the determination of proteins in pine needle extract was shown. A suitable method was developed for extracting proteins from pine needles. A total of 26 protein components, with a major protein being 55 kDa, was shown to be present in four pine trees

Table 3. **Amino acid composition[a] of WWP needle proteins**

Amino acid	WWP tag no.				
	3790	3790[b]	3795	3807	3866
Asp	11.7	12.1	12.2	13.2	11.6
Thre	6.6	7.1	6.7	7.7	6.8
Ser	15.4	14.8	12.0	11.1	13.0
Glu	15.3	15.2	16.2	14.5	15.2
Pro	5.1	5.4	4.4	5.6	3.5
Gly	**18.4**	**18.5**	**22.0**	**17.6**	**20.0**
Ala	9.1	8.3	7.3	8.8	8.3
Val	2.4	1.5	3.1	3.4	3.5
Met	0.9	1.0	0.7	1.1	2.0
Ile	2.9	3.2	3.3	3.6	3.1
Leu	3.9	3.4	3.7	4.9	3.8
Tyr	1.3	1.7	1.5	1.2	1.4
Phe	1.5	1.5	1.7	1.7	1.9
His	1.8	1.6	1.5	1.3	1.6
Lys	2.6	2.7	2.4	2.4	2.8
Arg	1.2	1.7	1.5	1.4	1.5

[a] Expressed as mole percent.

[b] Needles were not defatted.

examined. The presence of glycine rich proteins in the pine needles was demonstrated and the significance of this finding was discussed.

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CHLOROPLAST DNA IN WESTERN WHITE PINE: PHYSICAL MAP AND WITHIN-SPECIES VARIATION

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SUMMARY

A physical map of the chloroplast genome of western white pine (*Pinus monticola* Dougl. ex D. Don) was prepared and a variable Sal I site was located on the map. Chloroplast genomes with the variable site were significantly more frequent in interior than coastal trees.

INTRODUCTION

Recent expansion in applications of DNA technology has seen the techniques of molecular biology appearing in many fields, including studies of population structure. A survey of variation in chloroplast DNA of western white pine was undertaken to obtain information on population structure for the Pacific Forestry Centre's white pine improvement program. Similar methodology is now being used to look for molecular markers for rust races.

METHODOLOGY

DNA variation can be analyzed very simply by examining variation in recognition sequences for restriction enzymes. Restriction enzymes are highly specific DNA hydrolases named for their function in host-controlled restriction of bacteriophage growth. Individual restriction enzymes recognize a particular base sequence and hydrolyse the bond forming the DNA backbone within or near that sequence. Different restrictases recognize different sequences, for example, the enzyme Dpn II recognizes the sequence GATC. The discovery of restriction enzymes in 1970 provided a simple method for cutting duplex DNA molecules into discrete, reproducible fragments. They allowed direct comparison of DNA samples, since, if two samples digested with a particular restriction enzyme produce different sets of fragments they do not have the same base sequence. Differences in fragment size can be the result of single base mutations, additions, deletions, inversions or translocations.

The set of fragments produced by restriction digestion can be separated on agarose gels by electrophoresis. Individual fragments migrate according to their size, and will be well separated and can be clearly observed by fluorescent staining if the DNA molecule digested was a small one producing only a few fragments. However, if the DNA was of the size and complexity of a plant or fungal genome, electrophoresis and staining produce a smear of many overlapping fragments whose sizes intergrade. To compare DNA fragments from large molecules, the technique of Southern blotting, devised by E.M. Southern at the University of Edinburgh, is used (Southern 1975). This technique allows comparison of limited stretches of DNA, and is based on the complementarity of double stranded DNA. The complex DNA is digested with a restrictase, the fragments produced are separated by agarose gel electrophoresis, they are made single-stranded by treatment with alkali, and then immobilized on nylon membranes. The membrane is then hybridized in a solution containing the radiolabeled, single-stranded stretch of DNA to be compared. This "probe" DNA will hybridize only to fragments with sequences complementary to the probe sequence. The position of these fragments can be detected by autoradiography. These techniques were used to map genes and restriction sites on the chloroplast DNA of western white pine and to survey within-species variation at a restriction site.

CHLOROPLAST DNA

Western white pine in B.C. occurs in interior wet belt and coastal areas, with a separation of about 200 km in the drier area between them. Other B.C. conifers show physiological differentiation between interior and coastal populations which affects seed transferability. Long-term growth data on provenance effects are not yet available for white pine. Data on other biochemical characteristics such as terpenes and isozymes indicate there is very high within-population variation, making differentiation between populations difficult (Hunt and von Rudloff 1977; Steinhoff et al. 1983). Since chloroplast DNA is cytoplasmically, and frequently monoparentally, inherited, mutations arising in the chloroplast genome should spread in a clonal manner, (Palmer 1987) making population differentiation more likely than with nuclear mutations.

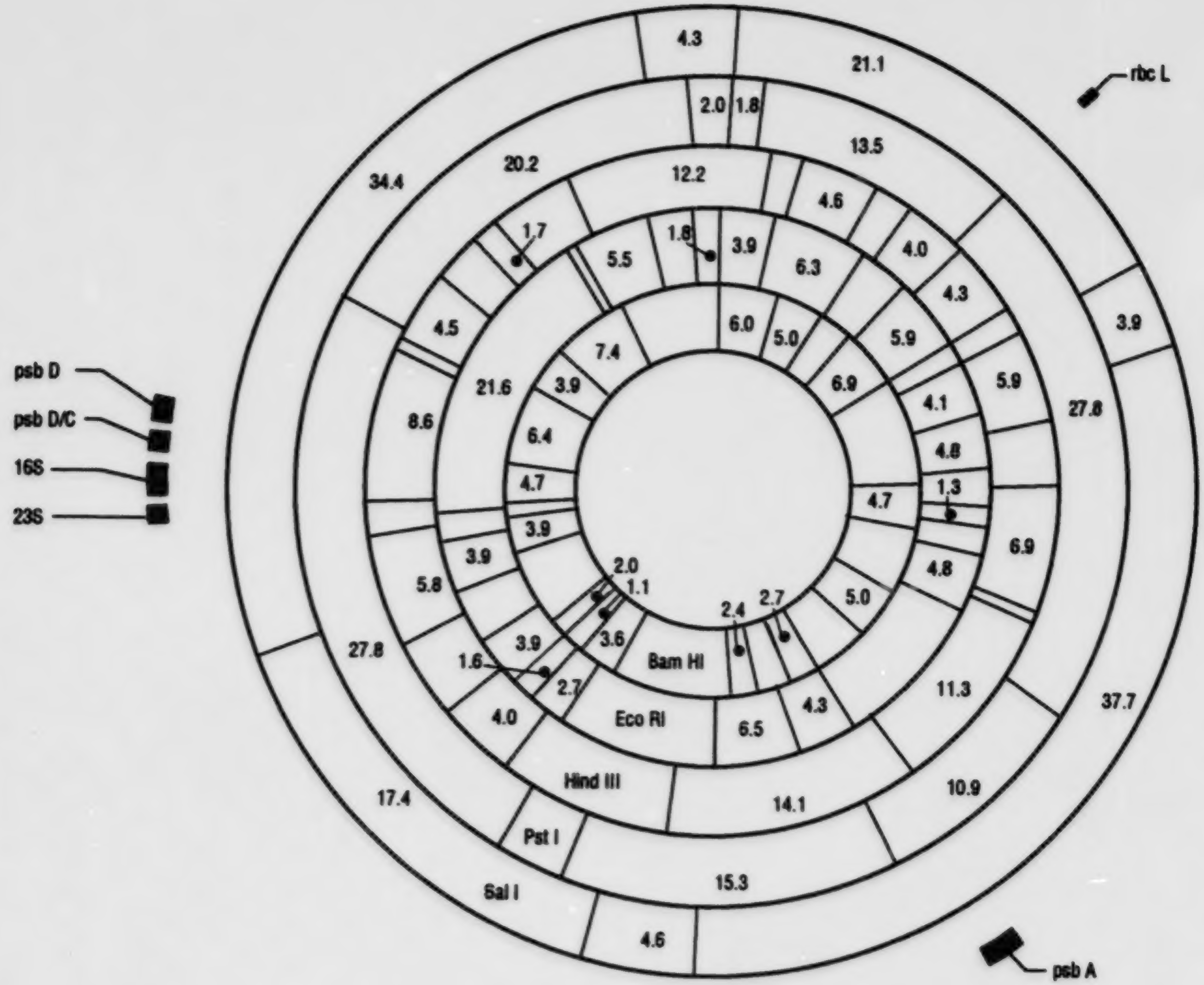
Restriction sites and genes on the chloroplast genome of white pine were mapped (White 1989a) (Fig. 1). Gene order is similar to that in radiata pine (Strauss et al. 1988), and the genome lacks an inverted repeat as do other conifers for which data are available (Strauss et al. 1988; Lidholm et al. 1988; Palmer and Stein 1986). A variable Sal I site was mapped on the 21.1 kb Sal I fragment on which the gene for the large sub-unit of ribulose bis-phosphate carboxylase occurs. A survey of the distribution of trees with chloroplast genomes containing this variable site showed they were significantly more frequent in interior than coastal populations (White 1989b) (Table 1). Chloroplast DNA in western white pine, as in other conifers for which data is available (Neale et al. 1986; Neale et al. 1988; Szmidt et al. 1987; Wagner et al. 1987), is predominantly paternally inherited, since 22% of open pollinated siblings had different chloroplast genomes. Some trees had both chloroplast genomes, indicating occasional biparental inheritance.

The results indicate a difference in the pollen cloud of interior and coastal white pine. Whether this correlates with biologically significant differentiation will be seen from results of provenance growth trials. The variant chloroplast genome provides an easily screened marker which predominates in interior populations.

CONCLUDING REMARKS

Differences in sizes of restriction fragments, (restriction fragment length polymorphisms or RFLPs), provide a relatively easily screened set of genetic markers. They have proven their usefulness as markers of human genetic disease, and in forensic science for identifying individual genotypes. In plant science they should be especially useful in providing genetic markers for species where monogenic morphological markers are infrequent, either because of a high degree of internal duplication or because of the organism's life cycle.

Linkage maps of RFLPs are being developed for several crop species which should allow rapid location of a new character with one or a few crosses. Maps are also being prepared for several species of phytopathogenic fungi (Michelmore and Hulbert 1987). Studies are continuing at the Pacific


4.3
21.1
rbc L
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1.7
1.8
5.5
3.9
6.3
4.5
6.0
5.0
5.9
5.9
7.4
6.9
psb D
psb D/C
16S
23S
21.6
3.9
8.6
4.1
27.8
6.4
4.8
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Bam HI
11.3
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Eco RI
6.5
4.3
Hind III
10.9
17.4
14.1
Pst I
15.3
Sal I
4.6
psb A

Figure 1. Restriction site map of *Pinus monticola* chloroplast DNA, showing restriction sites and positions of hybridizations with heterologous gene probes. Restriction sites are shown by radial lines. Bars marking the position of genes indicate the size of the probe used. Fragment sizes are given in kb.

Table 1. Frequency of trees with variant cp genomes

Coastal		Interior	
Population	Frequency	Population	Frequency
Bamberton	0	Barriere	0
Butchart Lake	0	Christina Lake	0.3
Butler Main	0	Heather	0.3
Manning Park	0	Lyall Creek	0.25
McKay	0	Mount Revelstoke	0.125
North West Bay	0.1	Perry River	0.4
Whistler	0.4	Salmon Arm	0.125
Woss/Davies	0	Valemont	0.1

Analysis

	Rank sum	Populations	Cases
Interior	87	8	76
Coastal	49	8	68
	Z = 2.00	P[Z] = 0.0456	

Forestry Centre to identify cloned probes suitable for detecting RFLPs in *Cronartium ribicola* J.C. Fisch., in order to examine geographic variability in the rust. Mitochondrial RFLPs can be used to study somatic fusion, while nuclear RFLPs provide markers for the majority of genes. Molecular markers, including RFLPs, are a powerful complement to existing genetic markers in rusts and pines.

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HISTOPATHOLOGY OF BACKCROSS PROGENY FROM (SHORTLEAF × SLASH) × SLASH HYBRIDS INOCULATED WITH FUSIFORM RUST

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ABSTRACT

Backcross progeny of (shortleaf × slash) × slash hybrids were sampled for histological study 1 year after control inoculation with fusiform rust, *Cronartium quercuum* f. sp. *fusiforme*. In general, the test progeny exhibited considerable susceptibility to the fusiform rust pathogen. Among the test progeny, three tissue-reaction types were observed: typically susceptible, pseudoresistant, and resistant. Typically susceptible samples exhibited macro- and microscopic anatomical features characteristic of fusiform rust in pine hosts. No host reaction or resistance to the development of the host-pathogen relationship was observed. Pseudoresistant progeny exhibited little or no macroscopic stem swelling and were characterized microscopically by localized infection zones of pathogen colonized tanned cells. These zones were bordered by periderm-like cells and adjacent parenchyma which were likewise colonized. This permitted escape of the pathogen from the zone and stimulated gall tissue deposition in the host which, in turn, produced the potential for continued gall development. Resistant reaction progeny were typified by limited areas of pathogenic tissue exhibiting degenerate rust hyphae buried in the xylem at or near the pith, and future development of this pathologic tissue was considered unlikely. The results indicate that judging progeny for rust resistance at 1 year after inoculation may not be a true evaluation of the reaction and future development of experimental progeny.

INTRODUCTION

This paper is a continuation of observations on the effects of fusiform rust, *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*, on the tissue systems of progeny from interspecies crosses and backcrosses of certain southern pine species (Jewell 1985, 1988; Jewell and Walkinshaw 1985).

MATERIAL AND METHODS

The progeny examined were from four shortleaf × slash parents (*Pinus echinata* Mill. × *P. elliottii* Engelm. var. *elliottii*) pollinated with bulk slash pine pollen. The progeny were artificially inoculated with fusiform at six weeks of age from seed (Jewell 1962). Control (uninoculated) progeny were from similar sources. Inoculated and control samples were collected for anatomical study one year following inoculation, prepared for serial paraffin sectioning, stained with orseillin BB/aniline blue (C.I. 26670/C.I. 42755) (Jewell 1988), and examined by light microscopy.

RESULTS

The control samples exhibited no cellular abnormalities associated with fusiform rust infection (Jewell 1962, 1988), and were anatomically typical for the genus *Pinus* (Esau 1962) (Fig. 1A).

Samples from inoculated progeny exhibited tissue reactions that were classified as one of three definable reaction-types: typically susceptible, pseudoresistant, or resistant.

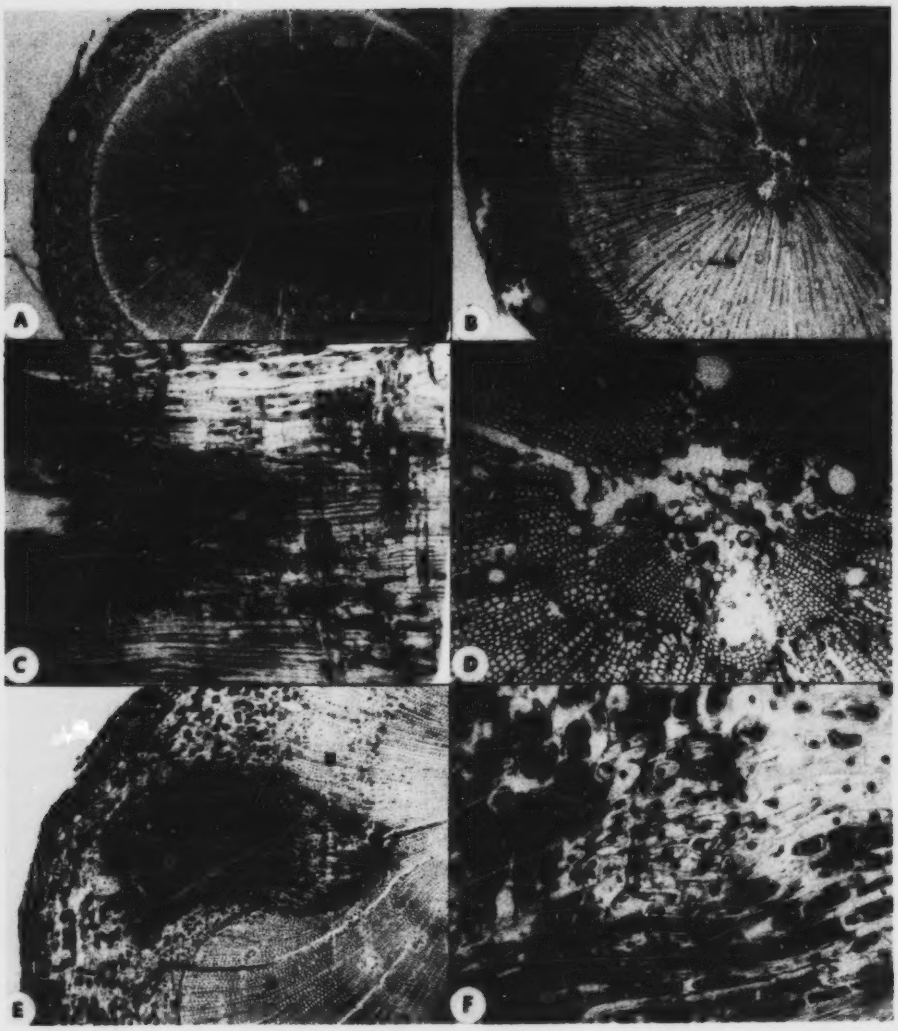
Typically Susceptible

Samples exhibiting this reaction-type were characterized by uniform abnormal anatomy throughout the entire circumference of affected stems and from the outer cortex to and into the pith (Fig. 1B). Typically fusiform rust hyphae were abundant in the gall tissue and appeared compatible with the host (Fig. 1C). The pathological tissue was similar to descriptions for gall anatomy of slash pine, loblolly (*P. taeda* L.) pine, and shortleaf × slash pine hybrids infected by fusiform rust (Jackson and Parker 1958; Jewell 1985; Jewell and Walkinshaw 1985; Jewell et al. 1962). The present samples exhibited, when compared to the normal, higher xylem rays which usually lacked ray tracheids, wider and longer ray parenchyma cells, hyperplasia of cortex and phloem where rays were more frequent, and shorter, wider xylem tracheids which frequently bore blunt ends. Reaction parenchyma (Jewell et al. 1962), near or at the pith, marked the internal limits of tissue abnormalities in the xylem, but, as reported for other pine-rust relationships (Jewell 1962, 1988; Jewell and Walker 1967) were absent adjacent to pith areas colonized by the pathogen (Fig. 1D). Colonization of pith areas by the pathogen was common (Fig. 1D), which contrasts with the fusiform rust-(shortleaf × slash) × shortleaf relationship (Jewell 1988). Whorls of rays and tracheids, common in rust galls on shortleaf pine and certain interspecies pine progeny (Jewell 1985; Jewell and Walker 1967), were lacking in the gall xylem.

In general, the samples observed exhibited compatibility between host and pathogen. There was no evidence that the host resisted the pathogen or the tissue changes associated with the progression of the host-pathogen relationship. Continued gall growth and development would very probably occur.

Pseudoresistant

Samples of this type differed considerably from the typically susceptible samples, particularly in the amount of stem circumference exhibiting pathological tissue. In transverse view, only portions of the stem were affected, while the remainder appeared normal (Fig. 1E). The affected stem areas, usually wedgeshaped, were isolated in the cortex, phloem, cambium, and outer xylem, and were typified as infection zones (Fig. 1E). These exhibited nonfunctional tanninized cells, apparently functional dark-staining parenchyma and parenchyma-like cells, and periderm-like cells surrounding the zone (Fig. 1E). The secondary cambium was interrupted where an infection zone extended into the outer xylem (Fig. 1E). Internally, the zones exhibited non- and functional rust hyphae and haustoria, which were associated with the parenchyma and parenchyma-like cells. There was no indication the pathogen was under stress as its characteristics in this parenchyma tissue appeared normal for fusiform rust (Jewell 1962) (Fig. 1F). Similar findings have been reported for other rust-pine host relationships (Jewell 1979; Jewell and Speirs 1976; Walkinshaw 1978). The pathogen in the zone tissue colonized the periderm-like cells bordering the zone as well as adjoining differentiating parenchyma (Fig. 1F). The presence of the rust in these tissue areas appeared to stimulate continued parenchyma development and production which, in turn, presented



Legend:
h = hypha(e)
s = haustoria
n = normal tissue
g = gall tissue
p = pith
r = reaction parenchyma
d = degenerate hyphae
b = xylem rays
c = secondary cambium

Figure 1. **A)** Transverse view of control progeny exhibiting normal pine anatomy (× 18); **B)** Transverse view of a typically susceptible progeny exhibiting wide xylem rays and reaction parenchyma surrounding the pith (× 18); **C)** Radial view of typically susceptible progeny exhibiting compatible and abundant hyphal development (× 75); **D)** Transverse view of pith of typically susceptible progeny exhibiting interrupted reaction parenchyma and corresponding colonized pith areas (× 75); **E)** Transverse view of a pseudoresistant progeny with an infection zone isolated in normal and gall tissue of the host. Note gall tissue developing adjacent to the zone (× 40); **F)** Radial view of hyphal extension from dark-staining cells bordering an infection zone in a pseudoresistant progeny (× 155).

a pathway for the escape of the pathogen from the infection zone. As growth of host and pathogen continued, typical gall tissue was differentiated in the vicinity of the zone (Fig. 1E). Such samples exhibited no regression of tissue or resistance to the production of gall tissue, and a reasonable assumption would be that, eventually, with host survival, a typical fusiform rust gall would develop. The time frame for such development is not known.

Resistant

Progeny samples in which the resistant-type reaction were observed exhibited no macroscopic abnormality. Internally, for the most part, normal pine anatomy was present throughout the stem cylinder. However, particular samples exhibited small xylem areas of abnormal cellular configuration at or in close proximity to the pith (Fig. 2A, B). The pathological tissue was parenchymatous in appearance and resembled tissue associated with rust infection in juvenile-primary slash pine tissue (Jewell et al. 1977, 1980). A heavily staining material was present intercellularly in the abnormal cells (Fig. 2A, B). This material appeared to be degenerate or dead hyphae as described previously (Jewell et al. 1977). The observations indicate that the combination of pathological tissue and associated degenerate hyphae in the vicinity of the pith represent an early rust infection of the juvenile plant. However, the establishment and progression of the pathogen was arrested, and normal host tissue growth subsequent to the infection effectively buried the pathological site in the xylem (Fig. 2A, B). No indication of escape or growth of the pathogen from the pathological site was observed. The host, presumably, would continue a normal pattern of growth to maturity.

DISCUSSION

The progeny from (shortleaf × slash) × slash crosses appeared quite susceptible to *C. quercuum* f. sp. *fusiforme* under the conditions of these experiments. The typically susceptible samples exhibited a gall anatomy characteristic of susceptible slash and loblolly pine (Jackson and Parker 1958;

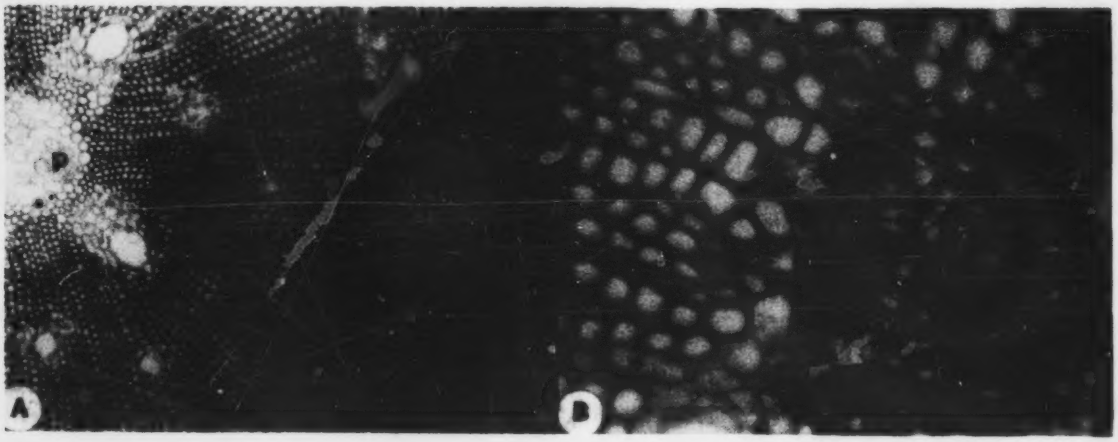


Figure 2. A) Transverse view of resistant reaction in xylem of resistant-type progeny (× 75); B) Enlarged view of A exhibiting abnormal parenchyma and degenerate hyphae (× 470).

Jewell et al. 1962). No evidence of host resistance to the pathogen was observed and typical gall development would be expected of similar plants under normal growing conditions.

The pseudoresistant reactions observed in particular samples were similar to tissue reactions reported previously in other pine hosts (Jewell 1988; Jewell and Speirs 1978; Jewell et al. 1980). The pathogen in the present work was initially isolated in the host tissue by a zone of tanned cells. These zones were usually adjacent to normal appearing host pine tissue, which composed the remainder of the stem cylinder. The host attempted to produce a periderm layer to isolate the zone, but the pathogen colonized the periderm-like cells and adjacent parenchyma. These colonized cells produced additional parenchyma. This differentiation resulted in the development of gall-type tissues in the host. Pathogen development and progression coincided with the deposition of the gall-type tissues. With progression from the zone area, the pathogen became associated with and in contact with the vascular cambium. This association produced a typical gall anatomy in localized areas of the host stem cylinder. This type of host-pathogen relationship, in the absence of any restrictions, was considered to have the potential, in time, to produce a viable fusiform rust gall.

The resistant reactions observed were strong indications that a definitive type of resistance to fusiform rust was present in certain of the experimental progeny. The complete isolation of the pathological tissue by normal host pine tissue deep in the xylem cylinder would appear to preclude escape of the pathogen from such areas. In addition, hyphae of the pathogen were observed to be degenerate and nonfunctional. Samples of this type potentially should continue a normal growth pattern.

The results indicate that the first year following rust inoculation may not be a true evaluation of the future performance of inoculated pine seedlings. Often progeny are judged for rust reaction by particular macroscopic symptoms at 1 year or less following control inoculation. A more definitive method might be to reexamine the progeny after 2-3 years or longer. The pseudoresistant samples in the present work would have very probably developed an active fusiform rust gall in time, if the host tree had lived, even though macroscopically the seedlings had originally, after 1 year, shown little if any indication of successful rust establishment.

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PENETRATION AND EARLY COLONIZATION IN BASIDIOSPORE-DERIVED INFECTION OF *MELAMPSORA PINITORQUA* ROSTR. ON *PINUS* STRUCTURAL AND ULTRASTRUCTURAL OBSERVATIONS

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INTRODUCTION

The initial steps of infection by Uredinales in the monokaryotic stage are as follows: 1) the formation of infection structures from basidiospores (germ tube, appressorium, penetration peg) and their subsequent penetration into uninjured host herbaceous organs and 2) the early colonization of host tissues by mycelium.

The literature on these topics (Littlefield and Heath 1979; Bushnell and Roelfs 1984) describes a direct penetration through the host epidermal cell wall for the monokaryotic stage and an indirect penetration through the stoma opening for the dikaryotic stage. However, as regards the monokaryotic stage, the different behavior of *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (Miller et al. 1980) compared to that of the cogeneric *C. ribicola* J.C. Fischer ex Rabh. (Patton and Johnson 1970) must be noted. These two rusts, both on species of *Pinus* genus, have direct and indirect penetration respectively.

The basidiospore-derived mycelium extends in the host tissue to form intercellular hyphae and intracellular terminal haustoria. The latter have different morphological aspects from those of the dikaryotic haustoria (for such differences in *M. pinitorqua* see Longo and Naldini Longo 1975). Sometimes the monokaryotic intracellular structures come out of the invaded host cells as reported in *Puccinia recondita* (Gold et al. 1979), *Melampsora lini* (Gold and Littlefield 1979) and *M. pinitorqua* (Longo and Naldini Longo 1982) during the first stages of colonization. Therefore, the initial steps of infection show a variable behavior depending on the different species, even if they are of the same genus.

The aim of this work was to observe the initial steps of the basidiospore-derived infection of *M. pinitorqua*, the heteroecious rust on some species of the genus *Pinus* and the *Populus* species of the *Leuce* section, in order to gain a better knowledge of the structure and ultrastructure of the rust monokaryotic stage.

MATERIALS AND METHODS

Primary needles of *Pinus pinea* herbaceous seedlings and elongating shoots of year-old *Pinus sylvestris* seedlings were collected at different times (between 7 and 94 hours) after artificial inoculation with germinating *M. pinitorqua* teliospores on leaves of *Populus tremula*. Small fragments of these organs were treated as follows, immediately after collection.

When using the SEM, the material was fixed in OsO_4 (1% in distilled water) with Photo-Flo 600 for 2 h at 4°C, dehydrated with a graduate series of acetone, critical point dried with CO_2 as transitional fluid, gold sputter coated and observed with a Philips SEM 505.

When using the LM and TEM, the material was fixed in 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.4 for 1 h and post-fixed in 2% OsO_4 in the same buffer for 3 h, dehydrated with the ethanol series and embedded in Epon 812. Serial sections were cut with a Reichert OMU3, alternatively thin (max. O.5μ) for LM and ultrathin for TEM. The former were stained with warm toluidin-blue and observed with a Leitz photomicroscope, the latter were stained with uranil acetate and lead citrate and observed with a Philips EM 300.

RESULTS

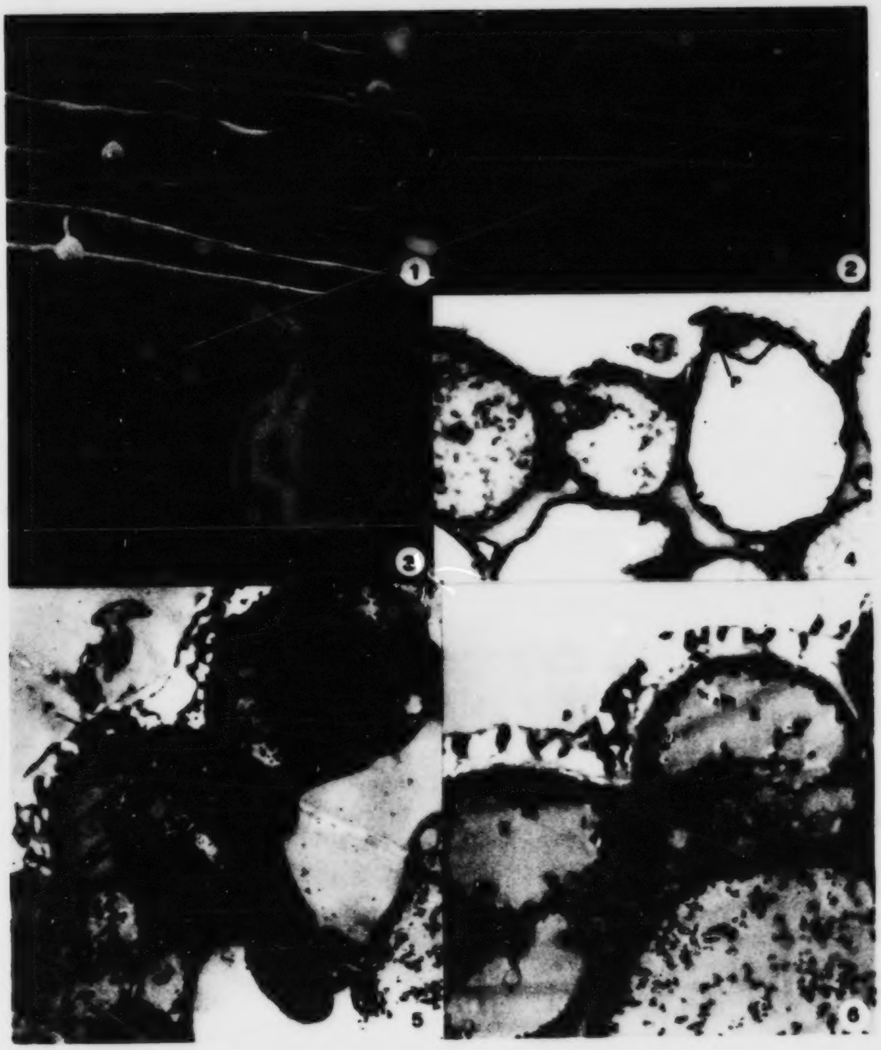
First of all, the rust preinfection phases on the host surface and the penetration into host tissues were considered.

On the epidermis of *P. pinea* primary needles and of *P. sylvestris* elongating shoots, observed using SEM, the subspherical appressoria were not very differentiated from the short germ tubes of the basidiospores, and adhered to any point of the epidermal cell wall (Figs. 1, 2). Here they produced typical hollows breached in the bottom (Fig. 3) probably due to the penetration peg.

On the cuticle of the herbaceous shoots of *P. sylvestris* observed using LM, the remnants of penetration structures produced by basidiospores were found at random on the periclinal epidermal cell wall. Then, the penetration peg breaching the cuticle and the cell wall, and the primary hypha inside the epidermal cell were found in succession (Fig. 4).

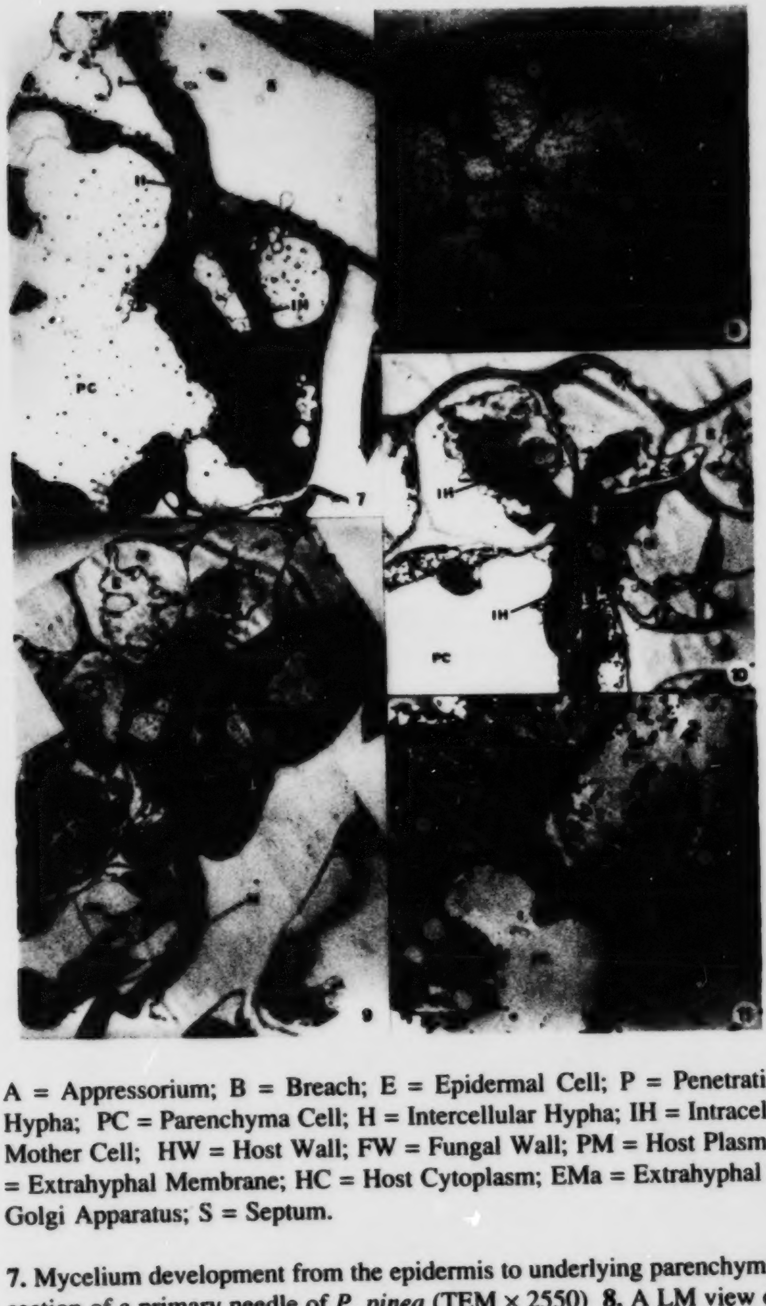
Under TEM the penetration peg usually appeared narrowed through the cuticle but was more expanded in the epidermal cell wall. The primary infection hypha which originated from the penetration peg extended inside the epidermal cell from where the colonization of host tissue started (Fig. 5).

In both materials observed, the primary infection hypha often showed transcellular growth from the invaded epidermal cell to the contiguous ones, giving rise to a tangential spreading (Fig. 6). At the same time this hypha grew downward into the underlying parenchyma producing intercellular hyphae and intracellular structures. The rust intracellular structures in the epidermis and in the parenchyma cells are here named "intracellular hypha" (*sensu* Gold and Mendgen 1984a) due to their behavior (Figs. 7, 8, 9). The parenchyma intracellular hyphae generally arose from intercellular mycelium cells acting as undifferentiated and nonterminal "mother cells" (Fig. 9); sometimes the same mother cell appeared to produce two intracellular hyphae in contiguous host cells (Fig. 10). Moreover, the parenchyma intracellular hyphae arose directly from mycelium transcellular growth, as in the epidermis. This was observed either when mycelium grew from the epidermis to underlying parenchyma (Fig. 9), or from one cell to another



Abbreviations: A = Appressorium; B = Breach; E = Epidermal Cell; P = Penetration; I = Infection Hypha; PC = Parenchyma Cell; H = Intercellular Hypha; IH = Intracellular Hypha; M = Mother Cell; HW = Host Wall; FW = Fungal Wall; PM = Host Plasma Membrane; EM = Extrahyphal Membrane; HC = Host Cytoplasm; EMa = Extrahyphal Matrix; G = Host Golgi Apparatus; S = Septum.

Figures 1-6. **1.** Germinated basidiospores on the epidermis of a primary needle of *Pinus pinea* (SEM × 1000). **2.** A germinated basidiospore with appressorium at the penetration point on the epidermal cell wall. Shoot of *Pinus sylvestris* (SEM × 5800). **3.** Particular view of an appressorium and the breached hollow produced on the epidermal cell wall. Shoot of *P. sylvestris* (SEM × 20 400). **4.** Penetration by a basidiospore germling through the periclinal epidermal cell wall. Shoot of *P. sylvestris* (LM × 1550). **5.** TEM view of a penetration peg and primary infection hypha in the same material as Figure 4 (× 3100). **6.** Hyphal transcellular growth in the epidermis. Shoot of *P. sylvestris* (TEM × 2950).



Abbreviations: A = Appressorium; B = Breach; E = Epidermal Cell; P = Penetration; I = Infection Hypha; PC = Parenchyma Cell; H = Intercellular Hypha; IH = Intracellular Hypha; M = Mother Cell; HW = Host Wall; FW = Fungal Wall; PM = Host Plasma Membrane; EM = Extrahyphal Membrane; HC = Host Cytoplasm; EMa = Extrahyphal Matrix; G = Host Golgi Apparatus; S = Septum.

Figures 7-11. **7.** Mycelium development from the epidermis to underlying parenchyma in a longitudinal section of a primary needle of *P. pinea* (TEM × 2550) **8.** A LM view of the same aspect of Figure 7 in a section of shoot of *P. sylvestris* (× 650) **9.** Hyphal transcellular growth from the epidermis to the first layer of parenchyma and an intercellular hypha producing an intracellular one in a host parenchyma cell. Shoot of P. sylvestris (TEM × 2450). **10.** Two intracellular hyphae produced by the same mother cell. Primary needle of *P. pinea* (TEM × 2750) **11.** Hyphal transcellular growth in contiguous cells of parenchyma. Shoot of *P. sylvestris* (TEM × 2750).

of the same layer of parenchyma (Fig. 11). The intracellular hyphae also extended, through transcellular growth, from the epidermal cells to the second layer of underlying parenchyma (Figs. 12, 13). More frequently they came out into the intercellular spaces, producing mycelium which again gave rise to intracellular hyphae in deeper parenchyma cells (Fig. 9). The intracellular hyphae sometimes appeared branched with one or several septa depending on their length. They usually had one nucleus per cell. Some of their interesting ultrastructural features were: 1) the penetration and exit sites through the host cell wall were very wide and here the fungal wall was continuous and clearly distinct from the host wall (Fig. 14); 2) a developing extrahyphal matrix was present; 3) the extrahyphal membrane bounded the matrix or was directly in contact with the fungal wall to some extent, and separated these from the host cytoplasm (Figs. 15, 16). The membrane was continuous with the noninvaginated host plasma membrane either at the penetration or exit sites of the intracellular hyphae (Fig. 14).

Around the parts of intracellular hyphae where the matrix was developing, a peculiar swarming of vesicles from host ER and Golgi (also in contiguity and continuity with the extrahyphal membrane) was present (Figs. 15, 16).

DISCUSSION

These observations (already briefly discussed by the authors Longo et al. 1988), do not focus on all the structural and ultrastructural characteristics of the initial steps of infection by *M. pinitorqua*. Notwithstanding this, they clarify some important aspects of that part of the rust life cycle.

A direct basidiospore-derived penetration was shown on *P. sylvestris* herbaceous shoots and on *P. pinea* primary needles. Nonetheless, on the latter, a definite connection between the exterior infection structures and the penetration peg has not been observed. Indeed, the basidiospore germlings grew in random directions on the surface of the inoculated organs; the appressorium and penetration peg differentiation occurred on the periclinal epidermal cell wall, but not at the stoma opening. These events were followed by formation of an intracellular infection structure within the epidermal cell, instead of an intercellular structure in the substomatal chamber which is typical of the indirect penetrations.

A direct penetration was described in detail for the monokaryotic stage of *Uromyces appendiculatus* var. *appendiculatus* on *Phaseolus vulgaris* (Gold and Mendgen 1984a, b), of *C. quercuum* f. sp. *fusiforme* on *Pinus taeda* (Gray et al. 1983) and on *Pinus elliottii* (Miller et al. 1980), and of *Endocronartium harknessii* on *Pinus contorta* (Hopkin et al. 1988).

On the other hand, we must add *Cronartium comandrae* on *Pinus banksiana* (Bergdhal and French 1984) to the reports of indirect penetrations by basidiospore germlings (i.e., *C. ribicola* on *Pinus strobus* cited as example in the introduction and some others reported by Gold and Mendgen 1984a). Considering *C. quercuum* f. sp. *fusiforme* an exception, Gold and Mendgen wrote that the indirect penetrations by basidiospore germlings usually occur on gymnosperms in response to the heavily cutinized thick-walled epidermal cells of needles. But first of all it must be pointed out that *M. pinitorqua* and more recently *Endocronartium harknessii* should be added to *C. quercuum* f. sp. *fusiforme* as examples of rust which directly penetrate gymnosperms. Furthermore: 1) a direct penetration is carried out by *M. pinitorqua* on herbaceous shoots and primary needles; by *C. quercuum* on hypocotyl, cotyledons, primary needles, shoots and secondary needles (Miller et al. 1980); and by *E. harknessii* on hypocotyl, cotyledons and growing shoots (Hopkin et al. 1988); 2) an indirect penetration is carried out by *C. ribicola* on cotyledons, primary and secondary needles (Patton and Johnson 1970); and by *C. comandrae* on hypocotyl, cotyledons and primary needles (Bergdhal and French 1984).

Abbreviations: A = Appressorium; B = Breach; E = Epidermal Cell; P = Penetration; I = Infection Hypha; PC = Parenchyma Cell; H = Intercellular Hypha; IH = Intracellular Hypha; M = Mother Cell; HW = Host Wall; FW = Fungal Wall; PM = Host Plasma Membrane; EM = Extrahyphal Membrane; HC = Host Cytoplasm; EMa = Extrahyphal Matrix; G = Host Golgi Apparatus; S = Septum.

Figures 12-16. **12.** Hyphal transcellular growth from the epidermis to the first layer of parenchyma. Shoot of *P. sylvestris* (TEM × 2250). **13.** The same hypha of Figure 12 in another section growing transcellularly to the second layer of parenchyma (× 2650). **14.** Particular view of the fungal wall and extrahyphal membrane in a hyphal transcellular growth. Shoot of *P. sylvestris* (TEM × 24 700). **15.** Intracellular hypha with developing extrahyphal matrix. Shoot of *P. sylvestris* (TEM × 16 600). **16.** Particular view of ER and Golgi apparatus in relation to the developing matrix of an intracellular hypha. Shoot of *P. sylvestris* (TEM × 24 550).

From these reports it may be seen that the same gymnosperm organs are penetrated directly or indirectly by basidiospore germlings depending on the rust species. Therefore, it may also probably depend on the rust species' mode of penetration into the organs usually infected in nature: i.e., a direct penetration by *M. pinitorqua*, *C. quercuum* f. sp. *fusiforme*, and *E. harknessii* on growing shoots (where too few or no stomata are present); and an indirect penetration by *C. ribicola* and *C. comandrae* on needles.

A knowledge of the basidiospore germling penetration patterns for each rust-host relationship is important in order to highlight particular host resistance mechanisms.

The intracellular hyphae of early colonization reported here were similar in structure, behavior and origin from intercellular hyphae to those of the monokaryotic haustoria already described for the Uredinales (Littlefield and Heath 1979; Bushnell and Roelfs 1984) and in particular for *M. pinitorqua* (Longo and Naldini Longo 1975, 1982). The origin of several intracellular structures from a single undifferentiated terminal mother cell into different contiguous host cells was also reported for *Endocronartium harknessii* on *Pinus banksiana* (Hopkin and Reid 1988).

On the other hand the intracellular hyphae growing in the host a few hours after inoculation also showed some particular aspects. Indeed, as for *U. appendiculatus* f. sp. *appendiculatus* (Gold and Mendgen 1984a), the infection hyphae frequently grew transcellularly either toward the underlying tissue or into the contiguous epidermal cells without producing intercellular mycelium. Moreover, this transcellular growth or the ability to escape from the invaded cells appeared peculiar not only to the infection hypha growing through the epidermal cell (as for *C. quercuum* f. sp. *fusiforme*, Gray et al. 1983), but also to the intracellular hyphae which subsequently spread into the underlying tissue (see *U. appendiculatus* var. *appendiculatus* too, Gold and Mendgen 1984b).

Another aspect of these intracellular hyphae was the extrahyphal matrix apposition at various degrees. In some cells, invaded a short time before, this could still be considered as not being apposed. Here, the extrahyphal membrane was directly in contact with the hyphal wall. The extrahyphal matrix development stage was characterized by a particular abundance of vesicles connecting the host organelles to the growing matrix. This was not observed when the extrahyphal matrix was thickened in older intracellular structures typical of an already established host-rust relationship (Longo and Naldini Longo 1975).

In conclusion, some characteristics of *M. pinitorqua* basidiospore-derived intracellular structures during the early colonization of host tissue, emphasize the differences between monokaryotic and dikaryotic rust stages described to date in the host cell-parasite relationship.

Therefore, it is useful to investigate whether or not such characteristics really represent a different degree of specialization and, thus, have a functional importance as regards the exchange of materials between the host and rust fungus.

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TEMPORAL AND SPATIAL MODELS OF COMANDRA BLISTER RUST INCIDENCE ON LODGEPOLE PINE IN SOUTHEAST WYOMING, USA

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ABSTRACT

Temporal and spatial risk prediction models were developed to provide silviculturists a means of better managing lodgepole pine stands (*Pinus contorta* Dougl. ex Loud. subsp. *latifolia* Engelm. (Critch.) in areas where comandra blister rust (*Cronartium comandrae* Pk.) incidence is high. Predictability of temporal occurrence of comandra blister rust infections was assessed by comparing the frequency distributions of potential basidiospore infection episodes from 40 years of weather data with those of canker ages. The total number of potential infection episodes ranged from 43 to 58 at three locations in Wyoming and Montana. Limited episodes (6-12 h duration) occurred at least once in 63-75% of the years, while moderate episodes (13-24 h) occurred in 30-40% of the years, and prolonged episodes (>24 h) only occurred in 8-10% of the years. Potential basidiospore infection episodes correlated well with actual canker ages since there were no significant differences between the cumulative frequency distributions of canker ages and weather episodes. The numbers of total potential infection episodes and cankers in 5-year age classes remained fairly constant with an infection rate of 0-1.5% per year indicating a continuous problem for managing lodgepole pine.

Spatial prediction of the rust was estimated by sampling: 1) potential habitats of the alternate host plant (*Comandra umbellata* (L.) Nutt. subsp. *pallida* (A.D.C.) Piehl) for shoot density and area occupied; and 2) the lodgepole pine forest for disease incidence and regular tree and site parameters. No strong correlations existed between disease incidence on lodgepole pine and any pine stand, or site parameters, or comandra plant parameters except location of the pine stand in relation to alternate host. A nonlinear regression equation ($R^2 = .72$) was developed that spatially describes potential rust incidence by utilizing average tree diameter and the distance lodgepole pine stands were from comandra plant populations.

COMANDRA BLISTER RUST: FACTS AND FANTASIES ABOUT COMANDRA HOSTS

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Comandra blister rust, caused by the fungus *Cronartium comandrae* Pk., is reported on more than 30 species of hard pines in many parts of the United States and Canada (Hiratsuka 1987; Johnson 1986). It is not known to occur outside of North America. It causes cankers much like those of white pine blister rust, commonly leading to death of seedlings and young trees, and dead tops in large trees.

In natural forests, comandra blister rust probably has greatest effect on lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.), especially in parts of Utah, Idaho, Wyoming, Montana, Alberta, British Columbia, and the Northwest Territories. Damage is of more local concern in native ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.) forests, but there are many reports of damage from California, Oregon, Washington, Idaho, Montana, and British Columbia. In the Ozarks of Arkansas and Missouri, the rust causes some damage to young shortleaf pine (*Pinus echinata* Mill.). In the east (Canadian provinces east of Saskatchewan and states east of the Mississippi River), comandra blister rust is usually more of botanical than pathogenic interest in native pine forests, but is known across the provinces to the Atlantic (Powell 1970) and in the Lake States in jack pine (*Pinus banksiana* Lamb.). This rust also occurs at low levels in other natural hard-pine forests in many areas.

In plantations, comandra blister rust is sometimes of greater concern. There are many examples of severely damaged ponderosa pine plantations in the western United States, and a few reports of heavily infected lodgepole pine plantations in western United States and Canada. Special concern exists when hard pines are planted outside their natural ranges where alternate hosts are present. Loblolly pines (*Pinus taeda* L.) planted a little north of their native range onto the Cumberland Plateau of eastern Tennessee were heavily impacted by comandra blister rust in the 1960s (Powers et al. 1967). More recently, 'Mondell' pine (a cultivar of *Pinus eldarica* Medw. from a plantation in Pakistan), a variety showing good promise for warm, arid sites, was found to be damaged by comandra blister rust in plantings near Prescott and Payson, Arizona (Maffel 1989).

Whereas the pine hosts of *C. comandrae* are very conspicuous plants, the alternate hosts are small, inconspicuous, and relatively little is known about them. Consequently, some misunderstandings prevail that I will try to clarify by separating fact from fantasy.

FIVE FACTS

Fact 1--The rust fungus *Cronartium comandrae* is known to alternate between hard pines and the two species of the Santalaceae, *Comandra umbellata* (L.) Nutt. and *Geocaulon lividum* (Richards.) Fern. The connection between the rust on pines and its alternate host was first proven in 1914 by inoculating shoots of comandra with aeciospores from the rust on ponderosa pine (Hedgcock and Long 1914). In controlled moist-chamber experiments, I have confirmed this connection many times with aeciospores from lodgepole pine inoculated onto comandra collected from northern Utah, and have successfully inoculated lodgepole pine a few times using telia-bearing shoots of comandra (Krebill 1968).

Buckleya districhophylla (Nutt.) Torr. is erroneously listed as a host in "Manual of the Rusts in United States and Canada" (Arthur 1934); collections upon which this was based were later shown to be a new rust fungus, *Cronartium appalachianum* Hepting (Hepting 1957).

Fact 2--Nomenclature is prone to change over time, adding confusion to the literature concerning the comandras. Currently, I prefer Piehl's nomenclature (Piehl 1965) for *Comandra umbellata*, which divides a morphologically diverse plant into four subspecies: *C. umbellata* ssp. *umbellata* (the sporophytic host of the rust in the Ozarks and the east); *C. umbellata* ssp. *pallida* (DC.) Piehl (the common bastard toad-flax host of the Rocky Mountain States and western Canadian provinces); *C. umbellata* ssp. *californica* (Rydb.) Piehl (occurs in California and Oregon); and *C. umbellata* ssp. *elegans* (Rchb. F.) Piehl (a Eurasian plant in the Balkans, unknown as a host for *Cronartium*). There is considerable variation within and intergradation between subspecies, causing confusion for those of us who like to place everything in neat categories. Much of the literature uses either separate species names for these taxons, or simply uses *C. umbellata*. All of the *C. umbellata* group in North America are known by the common names comandra or bastard toad-flax.

The taxon *Geocaulon lividum* is widely accepted, but much of the older literature used *Comandra livida* Richards. for the same plant. Northern comandra and northern bastard toad-flax are names used interchangeably for this plant, which is of widespread distribution across Canada and adjoining northern portions of many border states in the United States.

Fact 3--*Geocaulon lividum* is now known as a host of *Cronartium comandrae* in Montana. *Comandra umbellata* ssp. *pallida* has long been known as a host of comandra blister rust fungus in Montana, but that apparently has not been true for *G. lividum*, which occurs in small populations across the northern parts of Flathead and Kootenai Counties in northeastern Montana. In late August, 1988, Steve Wirt of the Flathead National Forest and I apparently made the first collections of the comandra blister rust fungus on northern camandra in Montana. We found the rust on northern comandra in the vicinity of Star Meadow, which lies about 30 km west of the town of Whitefish, in Flathead County. We also found comandra blister rust cankers on small lodgepole pine nearby. Above Star Meadow near Sheppard Creek, there was considerable damage in an area of about a hectare, in a recently thinned lodgepole pine plantation. Being late summer, aeciospores had long since been cast and there was evidence that *Tuberculina maxima* Rostr. had been active on the rust cankers. Collections (K-8803) of this new state record on northern comandra are filed in the National Fungus Collections in Beltsville, Maryland, and at Forest Service Research laboratories in Missoula, Montana, and Provo, Utah. These collections contain an abundance of well-developed telia on leaves and on occasional stems. The occurrence of comandra rust on northern comandra in northwestern Montana was to be expected, as it has long been known to occur on this host in similar sites in nearby Idaho, northeastern Washington, Alberta, and British Columbia.

Incidentally, *G. lividum* is listed as a "sensitive" plant in Montana (Reel et al. 1989), thus requiring special consideration in land management decisions by public agency personnel.

Fact 4--*Comandra umbellata* and *Geocaulon lividum* are plants somewhat similar in appearance, and both are hemiparasites with a wide range of hosts. Both comandra and northern comandra plants have perennial rhizomes, so that a single plant may be long lived, it may occupy many square metres of soil, and it may produce clumps of shoots or individual shoots. The cortex of rhizomes of *C. umbellata* ssp. *pallida* and *C. umbellata* ssp. *californica* usually contains a blue pigment that is a great help in learning to identify the plant in the field.

Small roots of comandras have been observed to form holdfast attachments that become embedded in roots of plants that they contact (Hedgcock 1915), forming an intimate parasitic union. The host range is very wide including many grasses, forbs, shrubs, and trees. Attachment to host roots apparently occurs very soon after germination of their nut-like seeds (Kuijt 1969), and perhaps is important to survival.

The aerial shoots of comandras usually sprout in early spring and persist until killed by fall frosts. Lower shoots of *C. umbellata* ssp. *pallida* are derived from underground buds, as their shoots are not persistent. Lower portions of the other subspecies tend to overwinter and form new shoots from basal buds in the spring. Aerial shoots generally attain heights of 15-30 cm, but some northern comandra shoots and some populations of comandra may commonly grow to 40 cm. Comandra and northern comandra are usually an inconspicuous part of the total vegetation on a site. Leaves of both species are simple, seldom over 3 cm in length, and somewhat pallid or light green. The flowers of both are primitive, rather small, and not very showy. Flowers of *C. umbellata* are greenish white and usually occur in small clusters at the top of the plant (Fig. 1). Fruits are globose to ovate, about the size of a small pea, and tend to be brownish or purplish brown at maturity. Flowers of *Geocaulon lividum* are greenish purple, solitary or very few in number, and develop from leaf axils along shoots. Fruits of both comandra and northern comandra are considered to be single-seeded drupes. The fruits of northern comandra are more fleshy and become a conspicuous orangish red, from a distance looking much like those of bearberry (*Arctostaphylos uva-ursi* Spreng.). Seeds are not produced in abundance by either comandra or northern comandra, so there is seldom a chance for quick and abundant invasion of new sites. Instead, the comandras seem to survive by their persistence, and by their ability to take advantage of any opportunities that come along at the expense of competing vegetation, by extending their rhizomes and producing new foliar shoots, and by drawing nutrients from their neighbours through parasitic connections.

Fact 5--Knowledge about the ecological habits of the comandra hosts can help us understand, predict, and avoid some of the more serious comandra blister rust problems in hard pines. For comandra blister rust to develop on pine there must be alternate hosts in the vicinity. Basidiospores produced on comandra and northern comandra are easily desiccated and killed in sunlight and dry air. Consequently, they most effectively spread with the wind only over fairly short distances and probably only rarely survive to infect pines over distances greater than a few kilometres.

In the Rocky Mountain States south of the distribution of northern comandra, the alternate host *C. umbellata* ssp. *pallida* almost always grows on open-steppe rangelands. These lands occur frequently just below the forest-covered slopes containing lodgepole pine, and less frequently are intermingled as openings in the forest, especially along rock outcrops and barren ridges. Soils of areas occupied by comandra are generally drier than those where forest trees, or especially where riparian vegetation or meadow species, prevail. Extremely large numbers of basidiospores can originate in some of the steppe areas where comandra is abundant, and comandra blister rust can be quite common in nearby lodgepole pine stands (Andrews and Harrison 1959; Boyd 1989). Recent field evidence indicates that pine infection can result from basidiospores covering distances of several kilometres (Jacobi and Geils 1990). In areas where pine losses are intolerable, it may be better forestry to favor nonhost tree species such as Douglas-fir, subalpine fir, or Englemann spruce, rather than continuing to regenerate lodgepole pine. Such areas are also poor places to consider planting other species of susceptible pines. In high-hazard areas where alternative species are not feasible, projected losses should be incorporated into future yield predictions. Fortunately, lodgepole grows in large continuous forests in numerous areas in the Rocky Mountains which do not have comandra habitat nearby; these are prime places for favoring lodgepole pine.

Figure 1. ***Comandra umbellata*** **ssp.** ***pallida*** **with a cluster of whitish flowers at the top of an aerial shoot.**

In many places in the west, small populations of comandra (either *C. umbellata* ssp. *pallida* or *C. umbellata* ssp. *californica*) grow within ponderosa pine forests, either in openings or under very light forest canopies. Under these conditions, it is likely that most infections originate from spores derived from comandra plants no more than a few hundred metres distant, as opposed to the longer-distance spread commonly suspected in lodgepole pine forests. Applying silvicultural practices that maintain and increase the density of canopy overstories as much as possible in ponderosa forests, or converting to nonsusceptible trees such as Douglas-fir, may be the easiest solution if comandra blister rust is a problem. Certainly such areas are not good places for planting susceptible pine species. *Comandra umbellata* ssp. *umbellata* is perhaps a little more tolerant of shade than the western subspecies of comandra, but it too tends to grow within the more open forests. Silvicultural practices that increase the density of forest canopies, and favoring or planting only nonsusceptible tree species, are logical ways to minimize comandra blister rust problems over large parts of North America.

Meinecke (1928) reported on waves of comandra blister rust in ponderosa pine in northern California, where it appeared that populations of comandra were held in check by rust infections as long as cankers on pines continued to sporulate. This apparently limited basidiospore production so that new pine infections tapered off. After aeciospore production ceased, the comandra presumably could again intensify and with a new influx of rust could set the stage for a new wave of pine infection. This interpretation deserves additional study where short-range basidiospore dispersal is suspected.

Geocaulon lividum tolerates shade better than *Comandra umbellata*, and it also grows in somewhat moister locations. Consequently, northern comandra is usually found within forest stands rather than in dry openings, which are more characteristic for comandra. Because basidiospores would be easily trapped by forest foliage soon after being cast from northern comandra, spread to pines would likely be restricted to very short distances. In the Montana site mentioned earlier, all the infected pines I saw were within a hundred metres of northern comandra plants. At the Montana location and at several locations in the Bowron Lakes area of British Columbia, where I recently observed the occurrence of northern comandra, this alternate host was growing beneath moderate or light forest canopies and especially near the edges of forest stands. In all the cases I have seen, northern comandra was in forest stands probably more than 100 years old.

If one were to correlate, with an adequate data base, the presence and abundance of northern comandra with a site classification scheme it may be possible to provide a very useful risk-rating technique that could be used to predict where the rust would be a problem to lodgepole pine plantations, and where alternate tree species should be favored. This would seem to be a useful approach to begin to minimize the comandra blister rust problem in the north.

Determining the response of northern comandra to various site preparation treatments might also be an important key to providing ways to reduce comandra blister rust damage in areas where lodgepole clearly is the best tree species and where northern comandra cannot be avoided. Certain treatments may increase the abundance of northern comandra, and others may reduce it. Carefully devised studies would be required, and attention should be paid to site and forest classification factors that also may influence results and applicability of findings. In my very limited observations at the Montana location, northern comandra was more abundant in parts of stands where harvesting had removed portions of the overstory. I could not find it in areas that had been clearcut and treated with prescribed fire to prepare the site for planting.

Improved range and fire management of sites in which comandra occurs also offers opportunities for reducing comandra blister rust problems, but to do so, we have much to learn. This brings me to my discussion of some fantasies concerning the comandras.

THREE FANTASIES

Fantasy 1--We understand the effects of range management on the abundance of *C. umbellata* ssp. *pallida*. James Mielke of the Intermountain Station was probably the first to postulate the importance of land management as an influence in the abundance of comandra and the resulting effects on comandra blister rust epidemiology (Mielke 1957). Following many years of observation and study, he put forth the idea that overgrazing of western ranges caused an increase in comandra, and that it could be reduced by better regulated grazing (Mielke 1961). Mielke's ideas are logical, in that the introduction of domestic ungulates less than a century earlier clearly had dramatically affected the vegetation, often reducing plant cover and increasing erosion, and thus possibly making more of the landscape suitable for comandra. Reduction of palatable grasses and increases in shrubs of low palatability were well documented in many places, but almost nothing was documented about the response of comandra to grazing.

To get a better grasp of the relation of grazing to comandra, range ecologist Bill Laycock and I examined transects inside and outside the few rangeland exclosures in the Idaho-Wyoming-Utah area for which we could find that comandra had been previously recorded. Our findings (Laycock and Krebill 1967) provided clear evidence that spring sheep-grazing caused comandra to decrease rather than to increase. Perhaps this is not surprising in that the young herbaceous shoots are selected by sheep along with other palatable herbs and grasses at that time of year. Incidentally, the same is probably true for deer, antelope, and elk, which now populate many of the rangelands of interest to comandra blister rust epidemiology, especially in the winter and early spring. Further studies should be designed to test the idea that spring grazing with sheep could be used to reduce comandra populations in areas where comandra blister rust is a concern.

Only one exclosure was in an area with cattle use; it had been originally established for Dr. Mielke for study of the effects of cattle-grazing. It was located in an area where cattle were trailed to more choice summer rangelands; elk used the area in winter. Measurements taken 9 years after plot establishment indicated that comandra shoots had approximately doubled outside the exclosure while remaining fairly constant within. This seems to add credence to Mielke's postulate and suggests that regulation of grazing may affect comandra populations.

Both the concept and the available information suggest that there is an opportunity to influence comandra blister rust through more intensive regulation of grazing of domestic and wild animals. However, since documentation of the intensities and effects of grazing are so meager, it is evident that additional research is needed to clarify the effects of grazing management on comandra ecology to the point needed to provide responsible management advice relative to comandra blister rust problems. Well-replicated plot studies with known intensities of grazing are needed, especially with respect to cattle-grazing. And it would be necessary to better define relationships between the amount of comandra and the likelihood of comandra blister rust infection in pines.

Fantasy 2--Herbicides offer a practical approach to reducing comandra abundance to a level that eliminates the comandra blister rust problem in pines. In the 1950s and 1960s, herbicides were seen as a practical tool to manipulate vegetation for improved productivity. During this time, the herbicide 2,4-D was widely used in the Rocky Mountain States to convert sagebrush fields into grasslands.

Observations from experimental herbicide plots by Mielke in the Teton National Forest in the mid-1950s[1] indicated that treatments with 2,4-D or 2,4,5-T and related herbicides were effective in killing a majority of shoots of comandra, but new shoots appeared in the following year and results were therefore unsatisfactory. Blaisdell and Mueggler (1956) reported light mortality of comandra (in one area it was "unharmed" and in a second it was classed as a "moderate" 33-66% kill) in operational applications of 2,4-D in eastern Idaho.

To better test the influence of contemporary range management practices on rusts, Roger Peterson of Intermountain Station, in 1962, established pairs of 4-m^2 plots in three sagebrush areas about to be operationally sprayed with 2,4-D in the Targhee National Forest in southern Idaho. He made shoot counts a few weeks before spraying and a year later. I made additional measurements on these plots over the following 8 years. The 2,4-D spraying was quite effective in killing sagebrush and other shrubs. For comandra, our measurements (Table 1) confirm earlier reports that 2,4-D can kill about half the aerial

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[1] Documentation of Mielke's study is in files of the Intermountain Research Station, Ogden, Utah.

Table 1. Numbers of comandra shoots on control plots and plots sprayed with 2,4-D in the Targhee National Forest. Plots were square, 2 m on a side, and were established in 1962 in and near sagebrush areas treated as part of normal forest operations

Plot number	Treatment	Before spray	Time after treatment			
			1 year	3 yrs	5 yrs	9 yrs
R-1	Control	299	519	--[a]	364	302
R-2	Sprayed	114	79	--[a]	--[a]	--[a]
TB-1	Sprayed	61	26	15	18	33
TB-2	Control	154	114	188	169	196
TB-3	Sprayed	34	12	17	39	72
TB-4	Control	84	82	103	106	201

[a] This plot was not found for remeasuring during that year. Plot R-2 apparently was on private land that was converted to a wheat field in 1963.

shoots, and we detected a subsequent increase in comandra in treated areas for several years following treatment. Throughout the measurement period, grasses dominated the sites although shrubs were becoming fairly noticeable within 5 years and occupied perhaps one-twentieth of sprayed areas after 9 years. Whether this type of treatment would have a substantial payoff in providing protection against comandra blister rust in pines remains questionable, but I suspect that a more effective and longer-lasting treatment would be more desirable.

I also explored the possibility of using several other herbicides, including Atrazine, Simazine, Prometone, Tordon, and Banvel-D. I used a factorial design with three replications for each of three application rates and three water controls for each chemical, all applied on 0.004-ha circular plots. Atrazine and Simazine applied in the spring at rates of 1.12-4.48 kg active ingredient per hectare killed more than half the shoots by late summer; however, the rhizomes apparently remained alive and produced nearly the same number of shoots in following years as before spraying. Prometone 25E applied at a rate of 122.4 L and Banvil-D at 2.24-8.97 kg active ingredient per hectare were more effective initially, but numerous shoots began reappearing in the following year. A year later, there were usually one-third to one-half the original number of shoots on plots most severely treated. Treatments with Tordon achieved a response similar to Banvil-D and Prometone when applied at rates of 2.45 and 4.48 kg active ingredient per hectare. Granular application of Tordon 22K applied at a rate of 8.97 kg active ingredient per hectare achieved the most persistent results, with less than 10% of the original number of shoots counted in plots 2 years after treatment. Since I was searching for a treatment that would be at least partially selective against comandra, and instead found that its associates were killed by these herbicides before comandra, this line of inquiry was suspended soon after it was begun. Should anyone ever follow up on this type of study, I recommend including some fairly large plots that clearly encompass the entire rhizomatous plants. In my studies using very small circular plots, it is possible that some of the shoots measured in seasons after treatment had originated from rhizomes that spread into the plot from surrounding nontreated areas.

Because of current environmental and social concerns, it is not likely that herbicides, even if they were effective and reasonably selective, would be widely used to limit comandra blister rust on public lands in the United States.

Fantasy 3--Fire control has caused comandra to increase. Based on observations of Dr. L. Roth of Oregon State University, an article reviewing the usefulness of fire for plant disease control (Hardison 1976) suggested that fire control had allowed comandra to increase, thus increasing the incidence of comandra blister rust. This is possible, but it tends to conflict with a few studies on rangelands of the Rocky Mountain States that included the influence of fire on *Comandra umbellata* ssp. *pallida*. Pechanec and Stewart (1944) summed up the results of many fires in southern Idaho, by noting that comandra is a species that spreads by rootstocks or root shoots and including it among species undamaged by fire. Blaisdell (1953) found no clear trend for comandra following fire in the sagebrush-grass ranges in southern Idaho, even though most rhizomatous forbs tended to increase after burning. I wonder if comandra's parasitism of its neighbors is a factor. Perhaps a hemiparasitic species would lose about as much in the way of parasitic nutrition as it gains from reduction in competition when a fire sweeps through its sites. Evidence from more recently burned areas helps little in clarifying the influence of fire. Kuntz (1982), in studies of prescribed spring burns in the Salmon National Forest of Idaho, indicated that comandra is among the plants least harmed and one that spreads most rapidly after burning, but he listed it as having decreased after burning. He speculated that more than 1-3 years of postfire measurement may be required to determine an increase. My own postprescribed spring fire observations are limited to two events in Montana in the early 1980s. In both cases a cursory look along the edges of the burns in the following year suggested that there was about as much comandra inside as there was outside the burned areas. Good quantitative studies of the effects of fire would be most welcome!

My interpretation of the available information suggests that fire does not have a strong influence on comandra in the Rocky Mountain States. There certainly is not enough evidence to agree with the inference of Navratil and Bella (1988) that comandra blister rust increased in the Rocky Mountain States in recent years: "...because of an increase in comandra plants, the alternate host, which previously had been depleted by ground fires...." This statement was based on Dr. Roth's observation reported by Hardison (1976), which was not based on Rocky Mountain conditions. The jury is still out with respect to Dr. Roth's observation as to its reality in coastal states where the comandra host is *C. umbellata* ssp. *californica* rather than *C. umbellata* ssp. *pallida*. Since the coastal form of comandra perennially retains basal portions of shoots and buds above ground, it is possible that it is more sensitive to fire than is inland comandra whose shoots annually die back to below the soil surface. Effects of fire on the two subspecies could be quite different.

As with other aspects of the ecology of the sporophytic hosts of comandra blister rust, our knowledge of the influence of fire has been gained mostly from limited observations rather than from well-designed experiments. If we are to provide better advice on ways to limit comandra, we must mount a more intensive program of research that includes quantitative studies of the influence of both wildfire and prescribed fire.

CONCLUSIONS

In the 75 years that it has been known that *Cronartium comandrae* alternates between pines and comandra, a fairly large body of information on the ecology of comandra has accumulated. Most of this information is based on observations by an astute cadre of botanists interested in parasitic plants and pathologists interested in reducing the impact of the disease in pines. All of the reported observations are useful, many withstand careful scrutiny, but some of the recorded information is misleading. I have tried in this paper to clarify some points of confusion, and to provide some additional observations on the ecology of comandras. Additional progress can be made through well-designed experiments to test hypotheses concerning the ecology of comandras and the role of these hosts in the epidemiology of comandra blister rust.

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TEMPORAL AND SPATIAL VARIATION AFFECTING FUSIFORM RUST HAZARD PREDICTION IN SLASH PINE PLANTATIONS IN THE SOUTHEASTERN UNITED STATES

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ABSTRACT

Fusiform rust incidence data in 5-year-old rust-susceptible slash pine plantations planted from 1961 through 1980 in the Coastal Plain of Florida and Georgia were examined to determine temporal and spatial variability. This variability was characterized within the region in successively smaller divisions of land, i.e., areas, tracts, and blocks, each having successively fewer numbers of associated plantations. Rust incidence in the region increased from 1961 to 1968 and decreased thereafter. Periods of high rust incidence occurred at 5-year intervals in 1963, 1968, 1973 and 1978. In both high and low rust incidence locations, percentage rust varied significantly among and within areas, tracts, and blocks. Significant variation in rust incidence was associated with year of plantation establishment. Rust incidence in adjacent plantations within blocks also varied significantly with year of planting. Although significant interactions between years planted and geographic locations were observed, rust incidence was consistently high in some years (e.g., 1968) and consistently low in other years (e.g., 1964). Higher rust incidence on slash compared with loblolly pine was related to planting years, not to differential species susceptibility. Temporal and spatial variation in rust incidence must be considered in predicting rust hazard at the plantation level.

INTRODUCTION

Fusiform rust, incited by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* causes major losses in intensively managed slash (*Pinus elliottii* Engelm. var. *elliottii*) and loblolly pine (*P. taeda* L.) in the southern United States (Powers et al. 1974, 1981; Dinus and Schmidt 1977). Regional rust incidence maps exist (Schmidt et al. 1974; Squillace 1976; Anderson et al. 1988) and fusiform rust-associated site factors are identified (May et al. 1973; Hollis and Schmidt 1977; Smith et al. 1977; Squillace et al. 1978; Froelich and Snow 1986). Prediction models using regional geographic rust hazard zones have been developed (Anderson et al. 1986; Borders and Bailey 1986) but within smaller geographic units or among plantations, temporal and spatial variation in rust incidence is poorly understood. Characterizing this variability is one prerequisite to defining rust hazard at the plantation level.

Schmidt et al. (1986) characterized temporal and spatial variation in fusiform rust incidence in the Coastal Plain of Florida and Georgia using forest inventory data from 5-year-old plantations. The authors quantified variability in rust incidence on a regional and subregional scale and reported the occurrence of areas of perennially high or low rust incidence. Subsequently, Schmidt et al. (1988) reported that site factors (primarily soil drainage and surface texture) were associated with rust incidence on a regional scale, but these associations were not evident among plantations within smaller land units.

The objective of this paper was to better define temporal and spatial variation in rust incidence among closely associated plantations, especially as this variation relates to rust hazard prediction.

METHODS AND MATERIALS

Rust incidence data (percentage of trees with a rust gall) were obtained from a plantation inventory at age 5 years in slash and loblolly pine in the Coastal Plain of Florida and Georgia. These data were described in detail by Schmidt et al. (1986, 1988). A portion of these data from closely associated (geographically) rust-susceptible slash pine plantations was examined for temporal and spatial variation in rust incidence. Temporal trends were examined by relating 5-year rust incidence to year of plantation establishment; annual rust incidence is not known.

To characterize spatial variability, the regional data from 1399 plantations on 48 500 ha were divided into successively smaller geographic units, i.e., areas, tracts, and blocks of associated plantations (Fig. 1). Within the region eight areas exist: five low rust incidence and three high rust incidence areas. Data from low rust incidence Area #1 (253 plantations, 10 970 ha) and high rust incidence Area #3 (215 plantations, 4900 ha) were examined. Areas were divided into tracts: sixty tracts were defined in the eight areas. Data from Tract #6 (56 plantations, 2740 ha) in Area #1 and from Tract #3 (72 plantations, 1390 ha) and Tract #5 (21 plantations, 344 ha) in Area #3 were examined. Tracts were further subdivided into blocks comprised of 3-6 adjacent plantations. Plantations ranged from 1 to 300 ha. Many areas, tracts and blocks were examined; those selected for analyses were representative and contained a sufficient number of plantations.

RESULTS AND DISCUSSION

Region

In the region, average rust incidence generally increased from 1961 to 1968 and decreased thereafter (Fig. 2). Factors responsible for these trends are not identified, but may be related to management practices. For example, decreasing rust incidence after 1968 could be related to improved nursery practices (Kelley and Cordell 1984) which reduce the number of rust-infected seedlings planted. Also, Schmidt et al. (1985) reported that rust-resistant genotypes were planted in high rust incidence locations (conversely, rust-susceptible materials were restricted to low rust-hazard locations) reducing overall rust incidence and perhaps rust inoculum.

Peak periods of high rust incidence occurred at 5-year intervals: 1963, 1968, 1973 and 1978 (Fig. 2). Years of high rust incidence are thought to occur periodically in the disease epidemic, and Froelich and Snow (1986) reported that infection varied by year and was dependent upon climatic factors. The regularity of the 5-year intervals in our data is difficult to explain since the data for each year of planting are an accumulation of infected trees at age 1 through 5 years. Thus, total percentage rust could have occurred from infection in one or more years.

Areas

Analysis of variance (Table 1) indicates significant differences in rust incidence among the eight areas as previously suggested by Schmidt et al. (1986). There was a significant effect of years

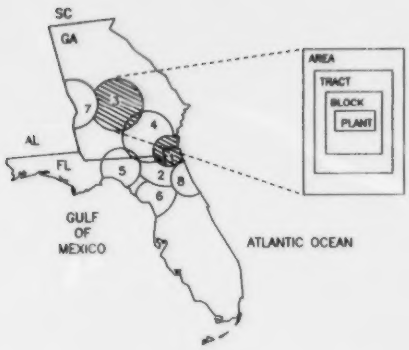

Figure 1. Location of eight areas within the Coastal Plain of Florida and Georgia in the southeastern United States where fusiform rust incidence data in 5-year-old pine plantations were obtained. The insert shows the subdivision of Areas #1 and #3 into tracts, blocks, and plantations.

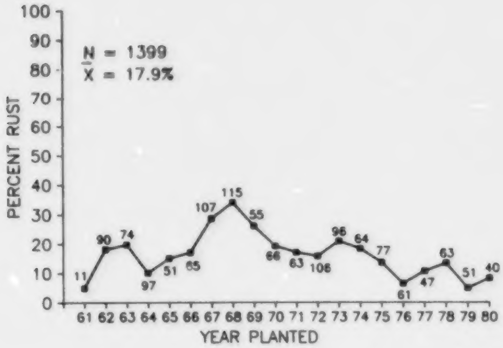

Figure 2. Average fusiform rust incidence by year planted in 5-year-old slash pine plantations in eight areas in the Coastal Plain of the southeastern United States. The numbers of plantations in each yearly average is shown.

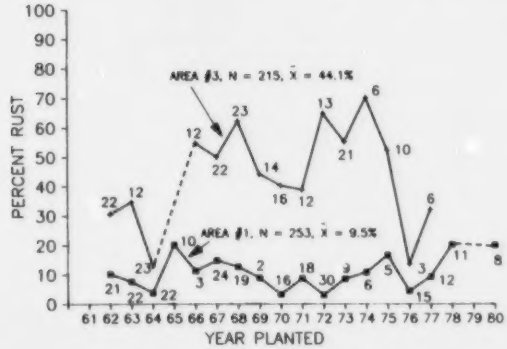

Figure 3. Average fusiform rust incidence by year planted in 5-year-old slash pine plantations in low rust incidence Area #1 and high rust incidence Area #3 in the Coastal Plain of the southeastern United States. The numbers of plantations in each yearly average is shown.

Table 1. Analysis of variance for temporal and spatial distribution in fusiform rust incidence[a] among 5-year-old slash pine plantations in the Coastal Plain of the southeastern United States

Source[b]	Degrees of freedom	Mean square	F ratio[c]
A. Among area within a region			
Model	113	4 379.5	47.9***
Error	1 285	91.4	
Area (A)	7	55 588.7	607.8***
Year planted (Y)	19	1 621.9	17.7***
A × Y	87	861.4	9.4
B. Among tracts within an area			
1) Area #1 (low)			
Model	99	112.8	4.6***
Error	131	24.5	
Tract (T)	12	365.2	14.9***
Year planted (Y)	17	217.3	8.9***
T × Y	70	44.1	1.8**
2) Area #3 (high)			
Model	47	1 328.1	8.5***
Error	163	156.9	
Tract (T)	4	301.2	1.9 NS
Year planted (Y)	14	3 888.9	24.8***
T × Y	29	233.5	1.5 NS
C. Among blocks within a tract			
1) Area #1, Tract #6 (low)			
Model	52	43.7	28.3**
Error	3	1.5	
Block (B)	21	47.0	30.5**
Year planted (Y)	11	103.0	66.8**
B × Y	20	7.6	4.9 NS
2) Area #3, Tract #3 (high)			
Model	67	510.6	47.0
Error	4	10.9	
Block (B)	43	616.1**	56.7**
Year planted (Y)	11	580.4	53.4
B × Y	13	102.5	9.4*

[a] Rust incidence is percentage of trees with a branch or stem gall at age 5 years.

[b] Areas, tracts and blocks are sequential geographic subdivisions (refer to text, Figure 1). Area #1 and Tract #6 are low rust incidence; Area #3 and Tract #3 are high rust incidence.

[c] NS = nonsignificant; *, **, and *** are significant at the 0.05, 0.01, and 0.0001 level of probability, respectively.

planted and of the interaction of years planted and areas within the region. These characteristics are evident in Figure 3, which relates average percentage rust to year of plantation establishment in Area #1 and Area #3. Rust incidence averaged 9.5% and 44.1% in Area #1 and Area #3, respectively. In both areas, plantations established in 1964 and 1976 had significantly less rust than most other years. Years of high rust incidence differ somewhat between areas (Table 2). With the exception of the amount of rust, the temporal trends in the two areas are similar.

Tracts

In low rust incidence Area #1, significant variation in percentage rust occurred among 13 tracts, among the years planted, and in the interaction of tracts and years planted (Table 1). In high rust incidence Area #3, percentage rust varied significantly among years planted, but there was no difference among the five tracts. Apparently, the large amount of variability of percentage rust in high hazard areas, as noted previously (Schmidt et al. 1986), precluded significant effects of tracts (location) and of the interaction of tracts and years planted. However, average rust incidence varied among tracts in other high rust incidence areas not included here.

Data from one tract each in a high (Area #3, Tract #3) and low (Area #1, Tract #6) rust incidence area (Fig. 4, Table 2) show trends similar to those of the area in which the tract is located. Plantations established in 1964 exhibit low rust incidence in both tracts. Tract #3, Area #3, exhibits low rust incidence in 1976 (Fig. 3). Years of high rust incidence varied somewhat between tracts, as was evident in the area data.

Blocks

Among Blocks

Percentage rust differed significantly among blocks and years planted in both low (Tract #6) and high (Tract #3) rust incidence tracts (Table 1). The interaction between blocks and years planted was significant only in high rust incidence Tract #3. The significant difference in rust incidence among blocks reflects the variation among aggregations of plantations within a tract. The significant difference among years planted reflects the effect of planting year, as previously observed at the tract level. Rust increased in some blocks but decreased in other blocks in the same tracts during the same period (Figure 5).

Within Blocks

Variation in rust incidence in adjacent plantations within a block is shown in Table 3. Six plantations planted in 1964 averaged 12.2% rust while 15 plantations planted in the same blocks in other years averaged 45.9% rust. The great variation in rust incidence among years in adjacent plantations is shown in Figure 5. In low rust incidence Tract #6 in Area #1 (Fig. 5A), percentage rust increased (Block 1117), decreased (Block 1120), or remained relatively constant (Block 1126) during the same period. In high incidence Tract #3 in Area #3 (Fig. 5B), rust among adjacent plantations decreased from 68% in 1967 to 41% in 1968 (Block 3020), but rust increased from 27% to 73% in Block 3002 during this time.

Table 2. **Average fusiform rust incidence[a] by year of planting in 5-year-old slash pine plantations within areas and tracts of low and high rust incidence in the Coastal Plain of the southeastern United States**

Year planted	Low rust, Area #1 (% rust)	Year planted	High rust, Area #3 (% rust)	Year planted	Low rust, Area #1, Tract #6 (% rust)	Year planted	High rust, Area #3, Tract #3 (% rust)
78	21.3 A[b]	74	68.9 A	67	14.3 A	72	67.8 A
65	20.2 A	72	62.7 AB	68	13.2 A	74	67.0 A
80	19.5 A	68	61.8 AB	62	12.5 A	73	57.5 AB
75	16.7 AB	66	54.7 BC	71	6.3 B	68	54.5 AB
67	15.3 ABC	73	54.3 BC	63	5.1 B	75	47.4 B
68	12.6 BCD	75	53.2 BC	72	4.1 B	67	45.8 BC
73	11.8 BCD	67	49.4 CD	73	2.3 B	71	45.4 BC
62	11.7 BCD	69	46.2 CDE	64	2.0 B	69	44.9 BC
74	10.9 BCDE	70	40.9 DEF	70	1.3 B	70	33.1 CD
77	10.2 BCDE	71	36.8 EF			62	29.4 D
66	9.1 CDE	63	34.7 EF			64	6.6 E
69	9.0 CDE	77	32.1 FG				
71	8.8 CDE	62	31.7 FG				
63	7.7 DE	76	22.2 GH				
76	4.5 E	64	13.1 H				
70	3.9 E						
72	3.9 E						
64	3.8 E						
Avg.	10.2		44.7		6.9		44.5

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[a] Rust incidence is percentage trees with a branch or stem gall at age 5 years.

[b] Rust means followed by common letters within a column are not significantly different from each other at the 0.05 probability level according to Duncan's Multiple Range Test. Only those years when ≥3 plantations were established were included in the statistical analysis.

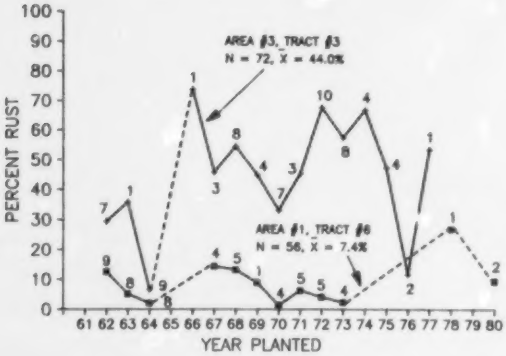

Figure 4. **Average fusiform rust incidence by year planted in 5-year-old slash pine plantations in low rust incidence Tract #6 (Area #1) and in high rust incidence Tract #3 (Area #3) in the Coastal Plain of the southeastern United States.** The numbers of plantations in each yearly average is shown.

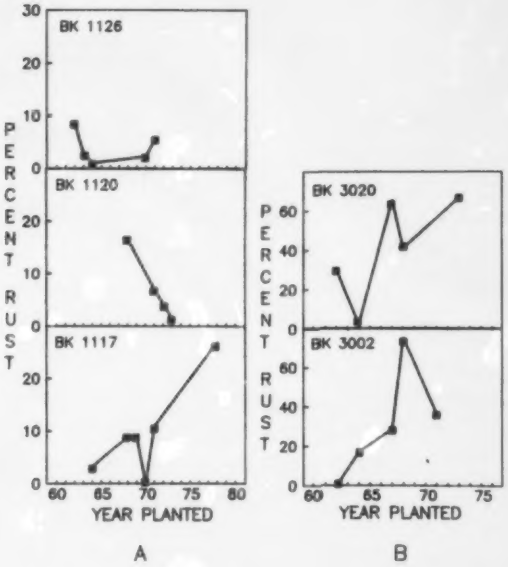

Figure 5. **Fusiform rust incidence by year planted in 5-year-old slash pine plantations within blocks of adjacent plantations in low rust incidence Area #1 (A) and in high rust incidence Area #3 (B) in the Coastal Plain of the southeastern United States.** Each data point represents one plantation.

Table 3. **Average fusiform rust incidence by year of planting in 5-year-old slash pine plantations within blocks in high rust incidence Tract #5 (Area #3) in the Coastal Plain of the southeastern United States[a]**

	% rust[c], by year planted									
Block[b]	1964	1962	1963	1967	1968	1969	1970	1971	1973	Avg.
3001	11.8		0.0				57.9			29.0
3002	16.9	0.0		28.6	73.1			36.4		34.5
3020	2.4	30.1		65.6	41.2				67.0	51.0
3023	15.9	34.5								34.5
3026	12.1		55.0							55.0
3027	14.3		61.4		79.8	58.7				66.6
Avg.	12.2	21.5	38.8	47.1	64.7	58.7	57.9	36.4	67.0	45.9**[d]

[a] Areas, tracts, and blocks are sequential geographic subdivisions (refer to text and Fig. 1).

[b] Blocks are contiguous areas in which plantations are adjacent or near to one another.

[c] Each observation represents percentage trees with a rust gall in one plantation at age 5 years.

[d] ** = significantly different according to Duncan's Multiple Range Test (0.05 probability level) from that in 1964.

Species

The importance of year effects for rust hazard prediction is demonstrated in Table 4. When slash and loblolly pines were compared in high rust incidence Area #3 without regard to year of planting, a significant difference between species was evident (as suggested by Schmidt et al. 1986) at the area (44.7% vs. 33.0%) and tract (43.4% vs. 26.2%) levels. When these species were compared using data from the same planting years, no statistical difference (28.1% vs. 26.2%) in rust incidence was observed. The high rust incidence in slash pine (52.5%) resulted from those years when only slash pine was planted.

CONCLUSIONS

Within the Coastal Plain region of the southeastern United States, the incidence of fusiform rust in predominantly 5-year-old rust-susceptible slash pine plantations varied significantly among and within areas, tracts and blocks, each representing geographic units of smaller areas and fewer numbers of more closely associated plantations. Much of this variability was associated with year of plantation establishment. Years occurred in high and low rust incidence areas and tracts when the percentage of trees with rust galls was consistently high or consistently low; for example, 1964 and 1976 were years of low rust incidence and 1968 often was a year of high rust incidence. Among blocks within the same tract, rust may increase, decrease, or remain constant during the same time period. Adjacent plantations often exhibit large differences in rust incidence between successive years.

Table 4. Average fusiform rust incidence in 5-year-old slash and loblolly pine plantations in a high rust incidence Area in the Coastal Plain of the southeastern United States

Location (years)[a]	Number of plantations	Average % rust[b]
Area #3 (all years)		
Slash pine	240	44.7
Loblolly pine	92	33.0 **[d]
Tract #3 (all years)[c]		
Slash pine	72	43.4
Loblolly pine	13	26.2 **
Tract #3 (years common)		
Slash pine	27	28.1
Loblolly pine	13	26.2 NS
Tract #3 (years different)		
Slash pine	45	52.5

[a] All years = all plantations regardless of year planted; years common = only the years when both loblolly and slash pine were planted; and years different = the years when only slash pine was planted (used to calculate average percent rust).

[b] Rust incidence is percentage trees with a branch or stem gall at age 5 years.

[c] Tract #3 is a geographic subdivision of Area #3, see Figure 1.

[d] NS = nonsignificant; ** = statistically significant at 0.05 probability level according to Duncan's Multiple Range Test: between species within locations.

Similar variation in rust incidence among years was also reported by Froelich and Snow (1986) who indicated that abundance and distribution of the alternate host (oak), climate, and susceptible pine growth were causal factors. Factor(s) responsible for temporal and spatial variation have not been determined for our data, and it is important to reiterate that rust incidence associated with year of planting developed over the 5 years from planting to inventory. However, these data suggest that temporal and spatial dynamics of fusiform rust incidence will make predicting rust incidence in individual plantations very difficult.

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BASIDIOSPORE INFECTION PERIODS FOR *CRONARTIUM* RUSTS ON JACK PINE IN MINNESOTA

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The southernmost range of jack pine (*Pinus banksiana* Lamb.) extends into Minnesota. This is a unique area because four *Cronartium* rusts are known to occur on hard pines, especially jack pine, and their alternate hosts. These rusts are *Cronartium coleosporioides* Arth. (stalactiform blister rust), *C. comandrae* Pk. (comandra blister rust), *C. comptoniae* Arth. (sweetfern blister rust), and *C. quercuum* (Berk.) Miyabe ex Shirai f. sp. *banksiana* Burdsall & Snow (pine-oak rust or eastern gall rust). The most important alternate hosts in Minnesota are *Melampyrum lineare* Desr. (cow-wheat), *Comandra umbellata* (L.) Nutt. ssp. *umbellata* Piehl (comandra), *Comptonia peregrina* (L.) Coult. (sweetfern), and *Quercus* sp. (oaks) for stalactiform, comandra, sweetfern, and pine-oak rusts, respectively. The natural basidiospore infection periods for these cronartium rusts are not well understood, but infection is reported to occur from late summer through early fall each year. However, our field experience suggested a much earlier and more prolonged period of basidiospore infection. The main objective of this study was to determine the basidiospore infection period for the cronartium rusts in this southern range of jack pine.

To determine when natural basidiospore infection of pine occurred, 1-month-old jack pine seedlings were exposed in the field to extensively infested alternate host plants of each rust. Seedlings were exposed to only one rust at each location, and a total of 200 seedlings were exposed to each rust (at each location) every 2 weeks from about mid-May through mid-October each year (1972-76). Precipitation events were recorded by date and amount for each period of exposure. All seedlings were maintained in the greenhouse and observed for rust infection for 9 months after field exposure. Seedlings were recorded as infected if either characteristic stem swellings or pycniospores appeared.

The following locations in east central Minnesota were used for this study.

Location	Rust	Exposure years
Barnum	Comandra blister rust	1974-75
Cloquet	Sweetfern blister rust	1973-76
McGrath	Comandra blister rust	1972-75
	Stalactiform blister rust	1972-75
Willow River	Pine-oak rust	1975-76
	Stalactiform blister rust	1974-75

In general, the basidiospore infection period for all four cronartium rusts was associated with precipitation events, but this study was not designed to quantify the precipitation-infection event. Also, the maximum amount of infection varied for each rust, location, and year of exposure, suggesting somewhat of a wave-year infection phenomenon for these rusts.

Basidiospore infection periods for stalactiform blister rust began as early as June 19 and ended as late as September 3. The peak period of infection was from about July 3 through August 15 each year (1972-75).

Basidiospore infection periods for comandra blister rust began as early as June 28 and ended as late as September 15. The peak period of infection, however, was from about July 15 to August 15 each year (1972-75).

Basidiospore infection periods for sweetfern blister rust began as early as June 6 (1976) and ended as late as October 5 (1973-75). The October ending dates were coincident with fall frost. The peak periods of infection were usually of long duration, beginning as early as July 21 and extending through September 3 (1973-76).

Basidiospore infection periods for pine-oak rust began as early as June 24 and ended as late as September 3. There appeared to be two distinct periods of infection, the first in late June and early July followed by a later period of infection during August (1975-76).

In general, the basidiospore infection period for each of these cronartium rusts is of longer duration than previously reported. These infection results, described above, should provide nursery managers and others with the necessary infection-period information to properly time the application of registered fungicides to periods of rust infection for susceptible pines in Minnesota and the surrounding region.

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EPIDEMIOLOGICAL EXPERIMENTS ON ITALIAN PROVENANCES OF *MELAMPSORA PINITORQUA* ROSTR. IN AREAS OF POSSIBLE OUTBREAK

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INTRODUCTION

Melampsora pinitorqua Rostr., "pine twist rust", is a parasite that occurs throughout Europe, northwestern Asia, and in some Middle East countries. It is a macrocyclic heteroecious rust which naturally produces its pycnio-aecial stage in several European two-needled pine species (*Pinus pinea* L., *P. sylvestris* L., *P. nigra* Arn., *P. pinaster* Ait., *P. halepensis* Mill.). Its uredial and telial stages occur in *Populus* species of the *Leuce* section (*Populus tremula* L., *P. alba* L., *P. canescens* Sm.).

The first record of the pine twist rust in Italy dates back to 1951 (Moriondo 1951). Since then, the rust has been observed in several zones throughout the country. The pine species which appeared naturally more susceptible were: *P. pinea*, *P. pinaster* and *P. sylvestris*. Twist rust infections were also found on *P. nigra* and *P. halepensis* (Moriondo 1951, 1954, 1957a, 1957b).

In Italy, the epidemic occurrence of the rust on pines, generally in the proximity of aspen, has been recorded mostly in the natural range of *P. pinaster*, i.e., in Liguria and northern Tuscany. It was also recorded on the southern border of the natural range of *P. sylvestris*, i.e., in the mountains of western Liguria. Other zones with outbreaks of the rust were in central and southern Italy. In these areas, both *P. pinea* on the coast and *P. pinaster* in the mountains of southern Tuscany are species which have been introduced (Longo et al. 1975).

The heaviest damage occurred on the sowing for planting of *P. pinea* and *P. pinaster* carried out in southern Tuscany. In some cases, the death of newborn seedlings caused the almost complete loss of sowing. Twist rust does not usually kill seedlings more than one year old. However, it causes serious distortions on the elongating pine shoots or, at worst, it makes thinner shoots wither. This withering of the shoots produces new adventitious shoots during the summer. Therefore, for seedlings more than one year old, rust infections delay development and distort several stems. Usually, the degree of infection increases progressively during the first four or five growing stages. Then it decreases and, when plants are more than 2 m tall, most shoots escape infection.

Observations in the natural environments carried out to date show that *M. pinitorqua* has spread, especially whenever there are stands of very susceptible pine species (*P. pinea* and *P. pinaster*) in the proximity of *Populus tremula* vegetation. It has rarely been found above elevations of 1000 m, i.e., on *P. sylvestris* regenerations. However, *M. pinitorqua* has not been found in the Vallombrosa forest (Tuscan Apennines), despite the presence of nurseries with several species of pine. Nor has it been found in the central Alps, despite large natural stands of *P. sylvestris* associated with the rust telial host *Populus tremula*. On the other hand, in these regions *M. larici-tremulae* was present on *P. tremula* and *Larix decidua*. Indeed previous studies (Naldini Longo et al. 1985) indicated that *M. pinitorqua* and *M. larici-tremulae* could be considered two distinct species of *Melampsora populnea* complex on *P. tremula*. They appeared different both in their morphology (even if limited to a few characteristics) and in their pathogenicity, despite a partially overlapping host range. More specifically, *M. larici-tremulae* is unable to infect *P. sylvestris*, in natural and artificial environments, while this is not the case with *M. pinitorqua*.

Following an examination of the different Italian provenances of the *M. populnea* complex, it was clear that these two rust species (*M. pinitorqua* and *M. larici-tremulae*) were separately present in two distinct areas. The former was present at Monticiano (southern Tuscany) and the latter at Bressanone (central Alps) and in the Vallombrosa forest. In the northern Apennines, at Pizzorne, Lucca, about 1000 m above sea level, where *Larix decidua* and *P. sylvestris* have been introduced into the natural area of *Populus tremula*, prior to 1975 only *Larix decidua* was found to be naturally infected by rust. However, since then the natural rust infection in this zone has also appeared on the artificial regeneration of *P. sylvestris*. The rust provenance of Pizzorne was then examined and it was found that *M. larici-tremulae* and *M. pinitorqua* were present together on the same *P. tremula* material (Naldini Longo et al.1988). On the other hand, it was uncertain whether or not *M. pinitorqua* was also present on the colder western exposure of this zone, where natural infections of the twist rust on *P. sylvestris* have not been observed.

The aim of the two trials treated in this work was to determine the possibility of *M. pinitorqua* outbreak in pine reforestation zones with different elevations and exposures from those of the rust's natural environment. This was also studied in relation to the presence of *M. larici-tremulae* in the same zones.

Teliospore germination tests and inoculations both on pycnio-aecial and uredial-telial hosts were carried out. The methods used were those described for *M. pinitorqua* by Longo et al. (1970, 1976) and Naldini Longo et al. (1985).

RESULTS AND CONCLUSIONS

In order to study the life cycle of the two rusts artificially developed in different environments from their natural ones, the first trial was carried out on uredial-telial stage development and the germinability period of the teliospores in relation to their formation and overwintering stations. Indeed, the epidemic occurrence of *M. pinitorqua* on pine derives, for the most part, from the amount of basidiospore inoculum produced by germinating teliospores.

The uredial-telial stage of the two rusts was artificially obtained on *P. tremula* sprouts inoculated separately in the same environment (experimental plots at Florence) with urediniospores produced from natural infections, i.e., respectively of *M. pinitorqua* from Monticiano and *M. larici-tremulae* from Vallombrosa. Furthermore, the uredial-telial stage of *M. pinitorqua* from Monticiano was obtained artificially in a typical *M. larici-tremulae* environment at Pizzorne. Then, telia from the two rusts from both artificial and natural infections overwintered together in Florence and at Pizzorne. Following this overwintering, the germinability period of teliospores and the pathogenicity of basidiospores on *P. sylvestris* and *L. decidua* (Table 1) were tested.

From the data the following conclusions can be reached:

1. The incubation periods of the artificially obtained uredial and telial stages were similar for the two rusts in the same station and for *M. pinitorqua* in the two different stations (respectively about 10 days and about 20 days).

2. The two rusts, artificially developed in different environments from their natural ones, maintained their typical germinability period of teliospores and their own pathogenicity (see Naldini Longo et al. 1985). Indeed, as can be seen in Figure 1, the *M. larici-tremulae* teliospores were already germinating abundantly at the end of February, while those of *M. pinitorqua* began to germinate in the middle of March and reached optimum rate during the month of April. Moreover, Table 1 shows there was no infection on *P. sylvestris* inoculated with *M. larici-tremulae*; however, many *P. sylvestris* seedlings inoculated with *M. pinitorqua* were infected. On the other hand, *L. decidua* seedlings inoculated with *M. larici-tremulae* were highly infected, while those inoculated with *M. pinitorqua* were less infected.

3. The germinability period of teliospores of the two rusts was affected by the overwintering station, but not by the formation station. In Figure 1, for *M. larici-tremulae* and for *M. pinitorqua*, on the same date, the germination intensity of the teliospores formed in their natural area and overwintered in Florence was similar to that of the teliospores formed and overwintered in Florence. Likewise, for *M. pinitorqua*, teliospores formed in their natural area and overwintered at Pizzorne, and those formed and overwintered at Pizzorne, showed the same germination intensity on the same date. Furthermore, the different germinability period of *M. pinitorqua* teliospores overwintered in Florence and overwintered at Pizzorne (i.e., late spring for a higher elevation about 1000 m) must be noted.

The second trial studied the artificial development of the two rusts at two exposures in Pizzorne (about 1000 m above sea level) and at different levels of elevation in the Vallombrosa forest.

Table 1. Artificial inoculations on *Pinus sylvestris* and *Larix decidua* with germinating teliospores of *Melampsora larici-tremulae* and *M. pinitorqua* formed and overwintered at Florence

Date of inoculation	Telial inoculum	Species inoculated	Number of seedlings	% of seedlings infected	Degree of infection
22.3	*M. larici-tremulae*	*P. sylvestris*	56	--	
		L. decidua	49	71.4	**[a]
12.4	*M. pinitorqua*	*P. sylvestris*	43	41.8	
		L. decidua	60	31.6	*[b]

[a] ** = Many infections per needle and many infected needles per seedling.

[b] * = 1-2 infections per needle and 1 to a few infected needles per seedling.

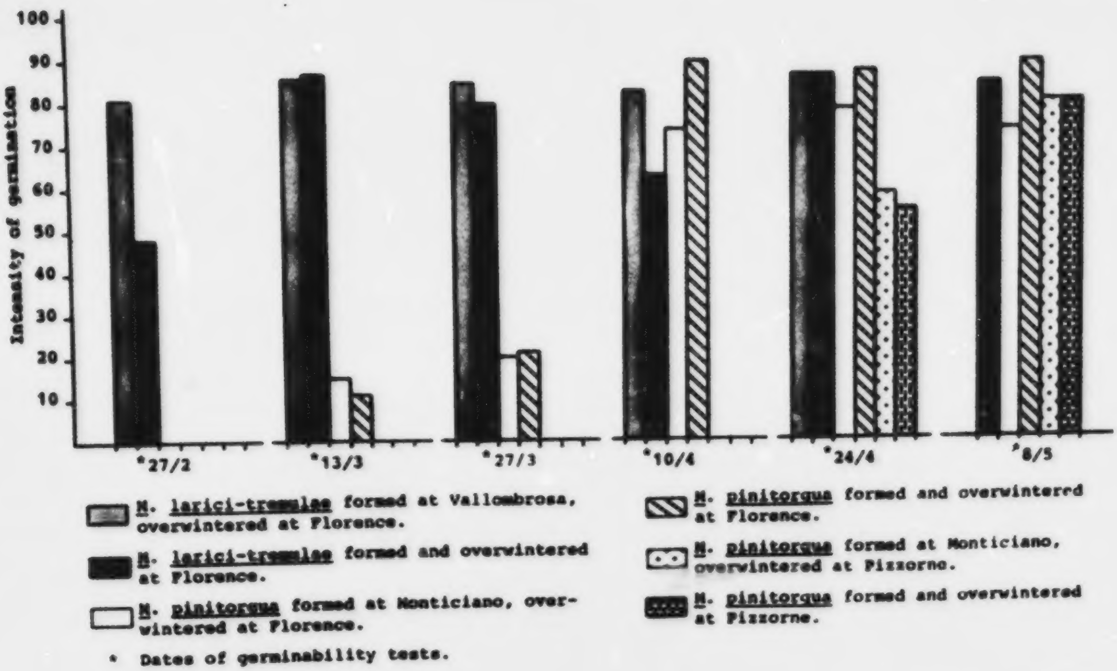

Figure 1. **Germinability tests on different sets of telia of *Melampsora larici-tremulae* and *Melampsora pinitorqua* depending on their formation and overwintering stations.**

We must remember that both these zones were natural areas for *M. larici-tremulae* (in Vallombrosa forest, *M. larici-tremulae* was present on *P. tremula* at about 800-1000 m above sea level). It was interesting to learn that *M. pinitorqua* could appear and survive in different stations in these zones.

The uredial-telial stage of the two rusts was artificially obtained on two sets of *P. tremula* sprouts for each level selected at Vallombrosa (700, 850, 900, 1100, 1200, and 1400 m above sea level). These were inoculated with urediniospores from natural infections, respectively of *M. pinitorqua* from Monticiano and *M. larici-tremulae* from Vallombrosa. The uredial-telial stage of *M. pinitorqua* was artificially obtained with the same procedure at the two exposures (south and west) of Pizzorne. Telia from natural infections of the two rusts and those obtained from artificial infections then overwintered together in each selected station. As controls, telia from natural infections of the two rusts also overwintered in their natural area (*M. pinitorqua* at Monticiano about 500 m, *M. larici-tremulae* at Vallombrosa about 1000 m).

After overwintering, the germinability period of teliospores from natural infections and the pathogenicity (on *P. sylvestris* and *L. decidua*) of the basidiospores from artificial infections were tested, and compared with the controls (Fig. 2 and Table 2).

The results from the second trial area are as follows:

1. The incubation period of the uredial-telial stage of the two rusts was similar in the different stations (10-15 days for the appearance of the uredinia and 20-25 days for the beginning of telia).

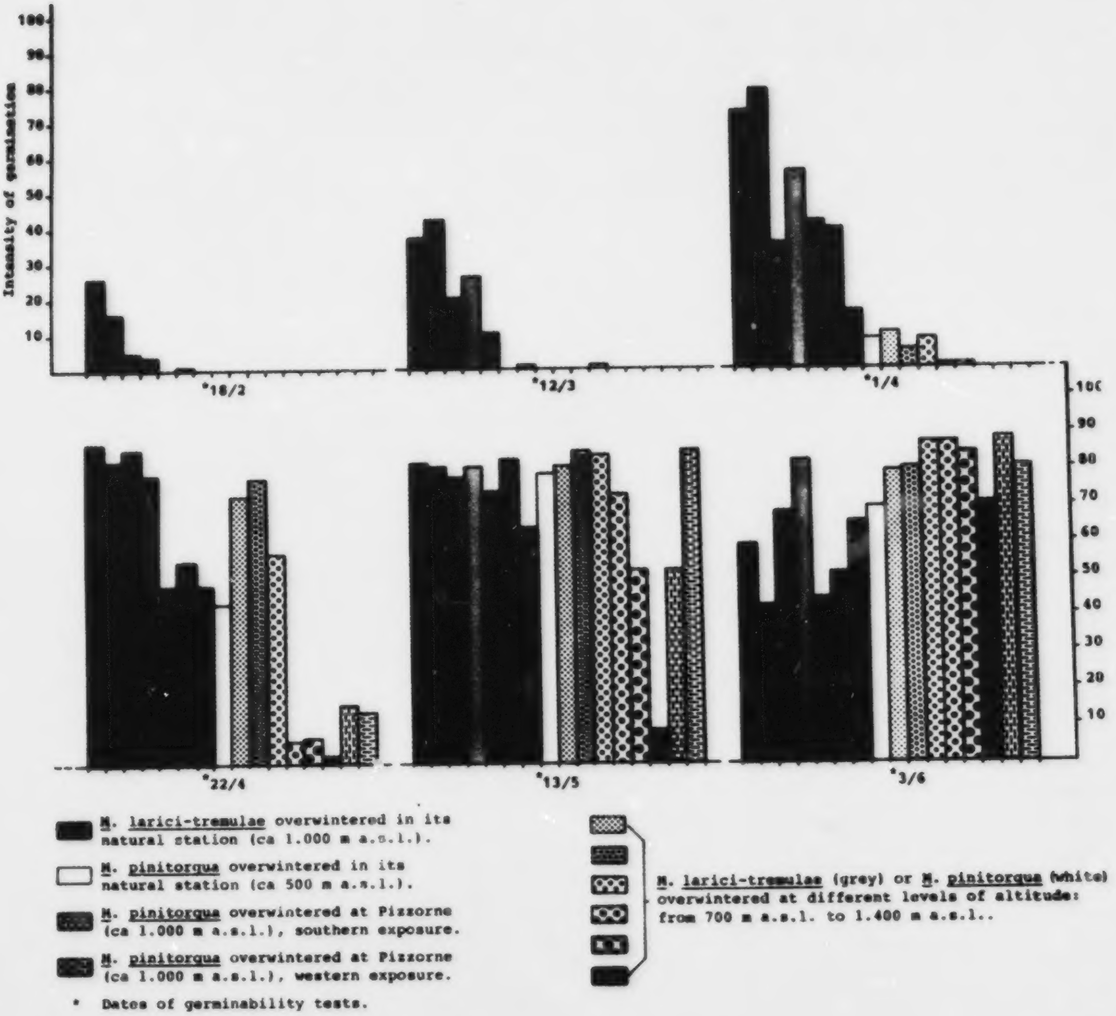

Figure 2. Germinability tests on telia of *Melampsora larici-tremulae* formed at Vallombrosa and *Melampsora pinitorqua* formed at Monticiano depending on different overwintering stations.

Table 2. Artificial inoculations on *Pinus sylvestris* and *Larix decidua* germinating teliospores of *Melampsora larici-tremulae* and *M. pinitorqua* formed and overwintered in their natural stations, at different levels of altitude in Vallombrosa, at different exposures in Pizzorne

Date of inoculation	Telial inoculum	Altitude in m a.s.l. or exposure	Species inoculated	Number of seedlings	% of seedlings infected	Degree of infection
9.4	*M. larici-tremulae*					
		V[a]	*P. sylvestris*	55	0.0	
			L. decidua	29	100.0	**[c]
		700	*P. sylvestris*	56	0.0	
			L. decidua	23	100.0	**
		850	*P. sylvestris*	53	0.0	
			L. decidua	28	100.0	**
		900	*P. sylvestris*	57	0.0	
			L. decidua	27	100.0	**
17.4	*M. larici-tremulae*					
		V	*P. sylvestris*	57	0.0	
			L. decidua	23	100.0	**
		1100	*P. sylvestris*	27	0.0	
			L. decidua	24	20.8	*[d]
		1200	*P. sylvestris*	57	0.0	
			L. decidua	26	100.0	**
		1400	*P. sylvestris*	23	0.0	
			L. decidua	26	100.0	**
25.5	*M. pinitorqua*					
		M[b]	*P. sylvestris*	50	30.0	
			L. decidua	26	50.0	*
		1200	*P. sylvestris*	61	21.3	
			L. decidua	31	64.5	*
6.6	*M. pinitorqua*					
		M	*P. sylvestris*	90	41.1	
			L. decidua	32	53.1	*
		southern	*P. sylvestris*	83	43.4	
			L. decidua	25	100.0	**
		western	*P. sylvestris*	50	24.0	
			L. decidua	28	17.8	*

[a] V = natural station of *M. larici-tremulae* at Vallombrosa.

[b] M = natural station of *M. pinitorqua* at Monticiano.

[c] ** = many infections per needle and many infected needles per seedling.

[d] * = 1-2 infections per needle and 1 to a few infected needles per seedling.

2. The germinability period of teliospores for both *M. pinitorqua* and *M. larici-tremulae* was later in spring depending on the altitude (Fig. 2). However, *M. larici-tremulae* maintained its advanced germinability period.

3. The two rusts, which were artificially developed in the selected stations, maintained their own pathogenicity (Table 2). Indeed, *M. larici-tremulae* overwintered at different levels of elevation in Vallombrosa did not infect *P. sylvestris* but infected at the maximum rate *L. decidua*. On the other hand, *M. pinitorqua* overwintered at a high level in Vallombrosa and on the southern and western exposures of Pizzorne infected the same percentage of *P. sylvestris* seedlings as of controls.

It appears that *M. pinitorqua* can survive in zones with elevation and exposure different from those of its natural environment, even with *M. larici-tremulae* on *Populus tremula* in the same area. Therefore, the *M. pinitorqua* outbreak in new mountain zones of pine reforestation is really possible.

Furthermore, in the higher zones, the delay in the germination period of teliospores in the spring could enable the twist rust to infect the later-shooting pine.

In spite of this, the absence of *M. pinitorqua* on pine in some areas of the Alps and Apennines could be due to specific phenological causes. These causes include the host shoots growth being out of phase, at any rate, with the period of rust basidiospore production, or *M. larici-tremulae* overcoming *M. pinitorqua* on *Populus tremula* leaves because of its considerably advanced life cycle.

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EPIDEMIOLOGY OF SCOTS PINE RUSTS IN POLAND

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SUMMARY

Scots pine diseases caused by the rusts *Melampsora pinitorqua*, *Endocronartium pini*, and *Cronartium flaccidum* occur in Poland over substantial areas of monospecific pine stands.

The epidemiology of the diseases is to a large degree dependent on the weather conditions in the given year. In the analyzed almost-30-year period, *Melampsora pinitorqua* occurred with various intensity with a culmination in the years 1961-63, 1966-68, and 1986-88. Occurrence of the disease is to a large degree dependent on the simultaneous occurrence of the second host *Populus tremula*.

In the past year there has been a significant growth of problems in Scots pine stands with *Endocronartium pini* and *Cronartium flaccidum*. On the average they affect an area of about 27 000 ha.

It appears also that the occurrence of rusts on pine seems to be closely associated with the degree of exposure of the forests to excessive gaseous industrial pollution.

INTRODUCTION

Stands of Scots pine occupy more than 70% of the forest area in Poland. In the majority of cases these are monocultures about 20-60 years old. For several years now, the sanitary state of these forests has undergone a systematic deterioration. Injurious abiotic factors, to which in the first place it is necessary to include high levels of air pollution, primarily with SO_2, cause a fast decline of stands and, as a result, a rapid degradation of forest sites. The phenomena of forest decline are intensified by weather anomalies in the form of long-term droughts, as a result of which in the weakened stands insect populations build up (as, for example, the gypsy moth explosion in the years 1979-86), and epiphytoses of dangerous fungal diseases occur. A detailed review of studies and methods used to improve the sanitary and health condition of Polish forests has been presented in the proceedings of the national symposium on "The biological reactions of trees to industrial pollution" (Siwecki 1987). Also in many other publications, reviews and reports of the condition of the present forest decline in Poland has been dealt with.

Among the dangerous fungal diseases, the importance of which constantly increases, the rusts of Scots pine caused by *Melampsora pinitorqua* Rostr., *Cronartium flaccidum*, and *Endocronartium pini* are currently particularly intensively studied and observed in the regions of Scots pine stands throughout Poland.

Also for a long time in other European countries, particularly in Italy, France, Sweden, and Finland, the stem deformation caused by *Melampsora pinitorqua* was and is intensively studied. Results of these various studies have been widely presented in numerous papers in proceedings of two IUFRO conferences organized in 1979: one in Suonenjoki, Finland (Weissenberg and Kurkela 1980), and the other in Florence, Italy (Powers et al. 1980). Also, at the IUFRO symposium organized in May 1989 in Poland (proceedings in print), several papers concerned the diseases in pine forests caused by an attack of *Melampsora pinitorqua*.

The results of investigation on *Melampsora pinitorqua* conducted in Poland have been presented in the papers of Siwecki (1974), Krzan and Siwecki (1980), Mlodzianowski and Siwecki (1975), Domanski (1976), Domanski et al. (1987), Domanski and Kowalski (1988). Relatively few investigations have been conducted on the other rusts of Scots pine, caused by *Cronartium flaccidum* and *Endocronartium pini*. From the annually performed evaluations of occurrence of the most important pests and infectious diseases conducted by the Forest Research Institute and from the prognoses of their occurrence, it appears that the rusts of Scots pine attack stands in large forest areas (IBL 1981-88). In 1988 the total area of stands endangered by serious fungal diseases in Poland amounted to 384 277 ha, of which 27 661 ha were Scots pine stands affected by rusts (IBL 1988).

The main purpose of the present paper is to analyze the occurrence of Scots pine rusts in Poland, particularly in the years 1981-88. An attempt was also made to relate the effect of weather conditions on the occurrence of these diseases. With respect to *Melampsora pinitorqua* the present occurrence of this disease has been compared with the earlier results in the years 1960-72 that have been presented in the work of Siwecki (1974).

OCCURRENCE OF *MELAMPSORA PINITORQUA* ROSTR.

In the years 1960-72 the area of young pine stands affected by the rust in the whole country was in the order of 3000-10 000 ha in different years (Siwecki 1974), demonstrating a declining trend in the following years. The smallest area affected by the disease was in 1981 and it amounted to 492 ha, after which there was an increase every year to 3004 ha in 1988 (Fig. 1). Presently the most affected stands are in the age class of up-to-20 years, though the occurrence of the disease on older stands is also reported.

Comparing more accurately the occurrence of *Melampsora pinitorqua* in younger pine stands in the years 1963-72 and in the years 1981-88, it is possible to say that the greatest danger occurs in the northeastern part of the country, lesser in central regions and the least in the southern montane regions (Fig. 2, 3). This distribution of the disease is determined by the more numerous occurrence in the north and east of the country, than in the south, of the second host of the rust, namely *Populus tremula*, a minor component of our forests.

From the presented data it appears that, in Poland, *Melampsora pinitorqua* attacks young Scots pine stands in those years in which there are favorable climatic conditions for the development of all phases of the pathogen on both hosts. After periods of local epiphytoses, the occurrence of the disease becomes relatively less and not so dangerous for the cultivation of Scots pine.

In view of the lack of detailed studies it is difficult to determine why there is an ever-increasing danger to young Scots pine plantations from *Melampsora pinitorqua* in regions strongly influenced by industrial emissions.

In the early seventies in European literature, the view dominated that the occurrence of rust fungi on Scots pine is less in stands endangered by emissions of industrial pollutants, particularly with

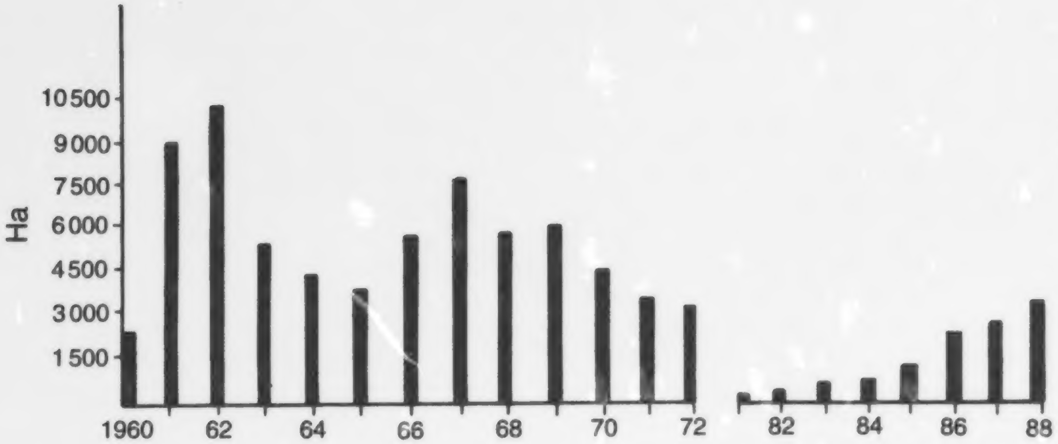

Figure 1. Area of young Scots pine trees affected by *Melampsora pinitorqua* in the years 1960-72 and 1981-88 in Poland.

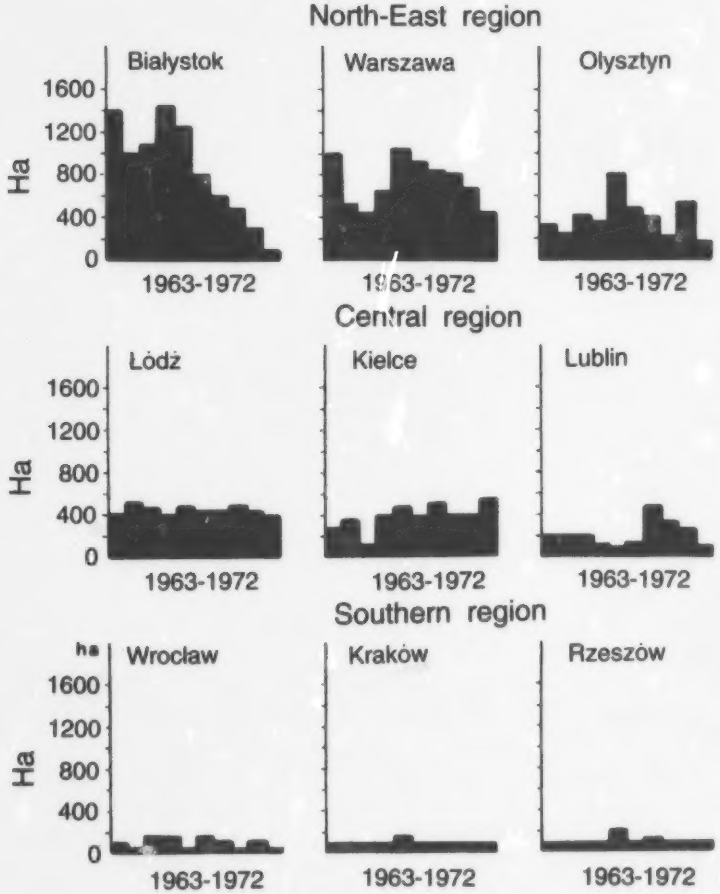

Figure 2. Area of young Scots pine trees affected by *Melampsora pinitorqua* in the years 1963-72 in three different regions in Poland.

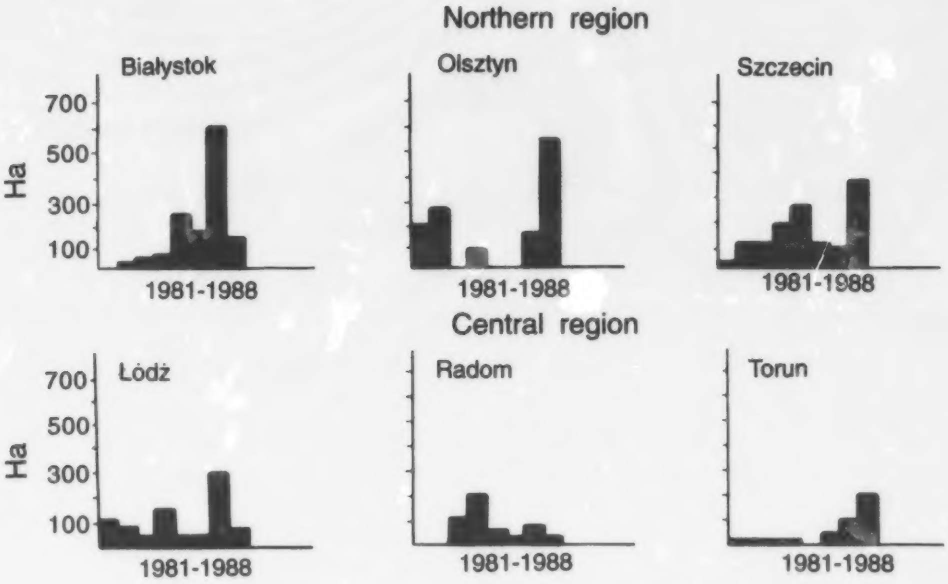

Figure 3. Area of young Scots pine trees affected by ***Melampsora pinitorqua*** in the years 1981-88 in northern and central regions of Poland.

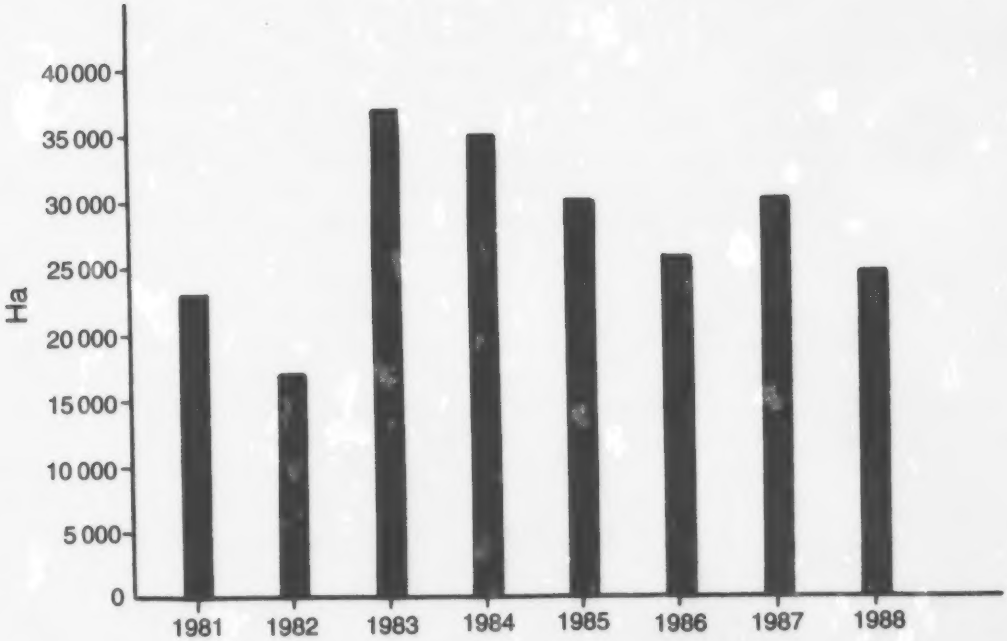

Figure 4. Area of Scots pine trees affected by ***Endocronartium pini*** and ***Cronartium flaccidum*** in the years 1981-88 in Poland.

substantial levels of sulphur dioxide. Domanski (1976) has, for the first time in Poland, described a case of epiphytotic attack on Scots pine in forests of the third zone of industrial pollution. According to Polish reports, in this zone there is a decline in annual volume increment amounting to about 70%, and in the second zone to about 50%.

More recent studies of Domanski et al. (1987) have shown an increased occurrence of *Melampsora pinitorqua* in Scots pine stands growing in industrial regions, primarily in zones II and III of industrial pollution intensity. One can suspect that results of these investigations may indicate that either new races of the pathogen developed in these regions or the effect is a synergistic response of such factors as industrial pollution, drought, insect pests, or other pathogens, which cause a rapid dying of the affected trees.

In the years 1985-86 in the southern part of Poland, an unusual dying of current annual shoots of Scots pine was observed on dry pine sites (Domanski and Kowalski 1988). The causal agents of this disease were the simultaneous occurrence of *Melampsora pinitorqua* and *Botrytis cinerea*. Local necroses of the bark and the associated twisting of shoots has been caused by *Melampsora pinitorqua*; while the further spread of the unusual necroses on the shoots, causing their rapid dying, has been caused by *Botrytis cinerea*.

OCCURRENCE OF *ENDOCRONARTIUM PINI* (PERS.) HIRAT. AND *CRONARTIUM FLACCIDUM* (ALB. ET SCHW.) WINT.

In Poland so far, we lack reliable studies on the biology and occurrence of these two serious Scots pine diseases and primarily of their accurate separation and identification. However, in recent years the importance of these rusts continuously increases, particularly on young pine stands growing on poor sandy sites where the rusts cause substantial damage. According to the data of the Forest Research Institute (IBL 1981-88) in the years 1981-88, the disease existed on an area from 17 177 to 35 271 ha. On the average, it annually affected 27 000 ha (Fig. 4). The lowest area affected was in 1982 when it was only 17 177 ha, increasing to 35 271 in 1983. This very dramatic increase in the area affected was undoubtedly caused by the drought that occurred in Poland in the years 1982-83. These diseases affect primarily even aged, monospecific Scots pine stands from 20 to 60 years old, growing on poor and dry sites. Annually, about 4% of the affected areas are younger, below 20 years.

The most affected regions in the country are the central ones and, to a lesser extent, the northeastern parts of Poland. In the years 1987-88 in the region of the greatest intensity of occurrence of these pathogens (central Poland), systematic treatments were performed in the spring in the affected stands, which consisted of removing affected and dying trees. As a result of these actions in the period discussed, the occurrence of the disease in that part of the country declined to an area of 3320 ha (IBL 1988).

ACKNOWLEDGMENTS

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RATE OF DEVELOPMENT OF WHITE PINE BLISTER RUST EPIDEMICS IN NORTH AMERICA

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ABSTRACT

From Maine to British Columbia, incidence of white pine blister rust on sapling to small pole-sized eastern and western white pines increased logistically. Values of r in high hazard areas ranged from 0.36 to 0.64 per year. Values of r in low to moderate hazard areas ranged from 0.08 to 0.26 per year.

INTRODUCTION

The forests of North America have incurred several serious epidemics of diseases caused by introduced pathogens or by pathogens of unknown origin. One of these diseases is white pine blister rust, caused by *Cronartium ribicola* Fischer. This fungus is a heteroecious rust that alternates between species of *Pinus* and species of *Ribes* and *Grossularia*. This fungus was introduced repeatedly into northeastern North America in the late 1800s and early 1900s. It was introduced into British Columbia in 1910. Since then the pathogen has spread throughout most of the commercial ranges of the eastern and western white pines, and white pine blister rust remains the major disease of these species.

Attempts have been made to examine the economics of control programs in different disease hazard areas (King et al. 1960; Marty 1967). However, in spite of the vast amount of research on this disease, little information is available on rates of disease increase to be expected in young stands. Quantitative measurements of rates of disease increase would form a mathematical basis upon which to estimate future losses and would be especially useful to those involved in simulation and modeling for management purposes. The following analyses were done to determine rates of increase of white pine blister rust based on published data from various localities in the U.S. and Canada.

MATERIALS AND METHODS

White pine blister rust on pine is a simple interest disease in any given year; that is, there are no repeating cycles on the pine host. If available published data on disease intensification is plotted, disease increase over a several-year period is somewhat logarithmic, however, the trend towards curvilinearity at high levels of disease incidence indicates that disease increase actually is logistic. Published data on rust incidence both on white pines and on *Ribes* were analyzed by van der Plank's (1963) formula to calculate the r values, that is, rate of disease increase per unit per year. Once average r values had been calculated for various time spans, they were substituted back into the formula and the times required for disease incidence to double from 10 to 20% were calculated.

RESULTS

Lachmund (1934) studied rust incidence on *Pinus monticola* Dougl. in permanent sample plots at Garibaldi, British Columbia. Plot 1 was located in the center of a *Ribes* patch, plot 2 was located on the margin of the *Ribes* patch and plot 3 was located 300 yards northeast of the *Ribes* patch. Values of r for tree mortality were: 1.29/yr in plot 1 from 1922 to 1924; 1.08/yr in plot 2 from 1923 to 1925 and 0.44/yr from 1925 to 1929 and 0.41/yr in plot 3 from 1925 to 1931.

Mielke (1937) studied rust incidence on *P. monticola* at Revelstoke, British Columbia. From 1922 to 1928 the r value for infection was 0.46/yr.

Posey and Ford (1924) studied rust incidence on *P. strobus* L. around a point source of *Ribes* at Kittery Point, Maine. The r value for tree mortality decreased throughout the course of the epidemic; from 0.50/yr from 1904 to 1908 to 0.19/yr from 1912 to 1916.

Snell (1931) studied rust incidence on *P. strobus* at Kelm Mt., Warren County, New York. The r value for mortality was 0.50/yr from 1923 to 1927 and 0.37/yr from 1927 to 1930.

Pomerleau (1961) studied rust incidence on *P. strobus* in Quebec. The r value for infection was 0.48/yr from 1958 to 1960; the r value for mortality for the same period was 1.44/yr.

Stewart (1957) studied rust incidence in four plots of *P. strobus* in northern Minnesota. The r values for infection were 0.64, 0.57, 0.50, and 0.47/yr. These calculations are "soft"; his data are based on 0.0% infection when the plantations were established and disease incidence 12 years later. To carry out the analysis, I assumed that 0.1% of the trees were infected the year of planting. This value may have been higher, in which case the r values are overestimated, or the trees may have become infected later, in which case the r values are underestimated.

Powers and Stegall (1971) studied rust incidence on *P. strobus* in North Carolina. The r value for mortality was 0.36/yr from 1946 to 1952 and 0.13/yr from 1952 to 1966.

Filler (1933) studied rust incidence on *P. strobus* in Vermont. The r value for mortality was 0.26/yr from 1925 to 1930.

Cafley (1958) studied rust incidence in *P. strobus* plantations in Ontario. The r value for infection was 0.08/yr from 1949 to 1956.

Mielke (1937) studied rust incidence on *Ribes lacustre* (Pers.) Poir. at Revelstoke, British Columbia. The r value based on percentage of plants infected was 3.03/yr from 1929 to 1930. The r value based on percentage of total leaves infected was 0.92/yr from 1929 to 1932. Disease was sufficiently severe to cause mortality of the *Ribes*, and disease incidence based on percentage of leaves infected decreased after 1932.

Snell (1931) studied rust incidence on *Ribes* spp. at Kelm Mt., New York. The r value for mortality was 0.37/yr from 1923 to 1926.

DISCUSSION

In stands of sapling and small pole-sized trees, infection and mortality rates should parallel one another although, because several years normally pass between infection and death, mortality should lag behind infection. One can assume that the mortality rate reflects a previous infection rate and calculate r values based on either infection or mortality. However, calculations based on mortality are likely to underestimate the true value of r because they do not take into account the high proportion of infection that occurs on lower branches (Mielke 1937; Snell 1929). These lower branches may be killed by shading before the fungus reaches the main stem (Snell 1929).

When the data consist of disease incidence at only two times (Filler 1933; Mielke 1937; Stewart 1957), one can calculate a logistic increase even though that increase may not have been logistic. I have included these calculations because I believe that this risk is minimal; in all other data sets (Cafley 1958; Lachmund 1934; Pomerleau 1961; Posey and Ford 1924; Powers and Stegall 1971; Snell 1931) white pine blister rust incidence did increase logistically.

The highest r values, 1.08, 1.29, and 1.44/yr (Table 1), are considerably greater than other calculated values; however, Lachmund (1934) stated that the rate of mortality in his plot 1 was the highest he had ever observed in North America. It is possible that these three rates are nonrepresentative of rates to be expected normally on high hazard sites.

The plot description of Filler (1933) indicates that he was working on a moderate hazard site (van Arsdel 1961). The r value was 0.26/yr. Although it cannot be proven, the change in r value at the Kelm Mt. site (Snell 1931) from 0.50 to 0.37/yr probably represents a change from high towards moderate hazard on this site due to one or both of two factors. First, as the stand aged, there were fewer infection courts due to natural death of lower branches and to the removal of trees in localized microclimates most favorable for the pathogen. Second, the disease had been so severe that extensive mortality of *Ribes* had occurred, thus considerably lowering the amount of available inoculum (Snell 1931). If this interpretation is correct, then values of about 0.20-0.40/yr represent rates to be expected on moderate hazard sites. Values less than 0.20/yr probably are representative of low-hazard sites. The low r value based on Cafley's data probably is due to lack of living branches low on the trees and low amounts of inoculum. Cafley (1958) noted that, as the canopy closed, the *Ribes* were shaded out. Based on these interpretations, Powers and Stegall (1971) were working initially with a high to moderate hazard site (r = 0.36/yr); as the stand aged and lower branches shaded out, this became a low hazard site (r = 0.13/yr).

If one excludes the three high values as being nonrepresentative and those values which appear to represent low or moderate hazard sites, the remaining 12 values range from 0.36 to 0.64/yr. These values are strikingly similar, a remarkable fact when one considers that they represent increase in disease incidence on two species of pine growing on very diverse sites with varied microclimates in widely separated areas of North America, spread from various species and densities of alternate host plants, and studies conducted by seven researchers or research teams over a span of 62 years.

Doubling times could not be determined for some data sets because the lowest disease incidence reported was greater than 50% or, as in the case of Stewart's data (1957), so close to 50% that the calculations would be meaningless. The time required for disease incidence to double from 10 to 20% varied from 0.8 to 1.7 years for high hazard sites and 3.1 to 10.1 years for moderate to low hazard sites.

Values of r of 0.4-0.6/yr may seem low for a disease caused by an introduced pathogen attacking a host with very low or negligible resistance. Indeed, these values are low when compared to

Table 1. **Infection rates (r/yr) and years required for disease incidence to double from 10 to 20% (Δt) for white pine blister rust epidemics on pine and *Ribes* at various locations in North America**

r/yr	Δt	Author of data	Comments	Location
PINE				
1.44	-	Pomerleau	Mortality	Quebec
1.29	-	Lachmund	Plot 1	British Columbia
1.08	0.8	Lachmund	Plot 2, early	British Columbia
0.64	-	Stewart	Area 4	Minnesota
0.57	-	Stewart	Area 2	Minnesota
0.50	1.6	Posey & Ford	Early	Maine
0.50	1.6	Snell	Early	New York
0.50	-	Stewart	Area 1	Minnesota
0.49	-	Stewart	Area 3	Minnesota
0.48	1.6	Pomerleau	Infection	Quebec
0.46	1.7	Mielke		British Columbia
0.44	-	Lachmund	Plot 2, late	British Columbia
0.41	-	Lachmund	Plot 3	British Columbia
0.37	-	Snell	Late	New York
0.36	2.2	Powers & Stegall	Early	North Carolina
0.26	3.1	Filler	Moderate hazard	Vermont
0.18	-	Posey & Ford	Late	Maine
0.13	-	Powers & Stegall	Late	North Carolina
0.08	10.1	Cafley		Ontario
RIBES				
3.03	-	Mielke	Infection of plants	British Columbia
0.52	-	Mielke	Infection of plants	British Columbia
0.37	-	Snell	Mortality	New York

those calculated for chestnut blight or Dutch elm disease (Merrill 1967). These values seem reasonable, however, when one considers that this disease is caused by a heteroecious rust with no repeating cycles on the pine, that the fungus must find favorable conditions for infection on two different hosts at two different times of the year, and that environmental conditions favorable for infection of the pine are somewhat narrow.

Because the fungus has a repeating cycle on the *Ribes* foliage, the disease is a compound interest type on *Ribes* in any given year; however, the data of Mielke (1937) show that the disease also may be compound interest from year to year on *Ribes*, at least until 100% of the plants are infected and defoliation is sufficiently severe that the plants begin to die. The r values for disease on the *Ribes* are so few and disparate that no conclusions can be attempted. The data of Mielke (1937) and Snell (1931) dispel the myth common among forest pathologists that *C. ribicola* is not damaging to the *Ribes* host.

It is interesting to note the opposite interpretation that may be given to the same data. Posey and Ford (1924) recorded that 0.9% of the population on their plot became infected from 1901 to 1904, and 18.3% became infected from 1913 to 1916. They concluded that the average annual infection rate had increased 20-fold during the course of the epidemic; however, the r value declined steadily from 0.50/yr early in the epidemic to 0.19/yr late in the epidemic. Thus, disease incidence was doubling every 1.5 years early in the epidemic and only every 7.7 years late in the epidemic. Hence, the infection rate actually declined to about 20% of the initial rate.

These data illustrate that a disease may be a simple interest disease in any given year but may be compound interest over an extended period of years. The epidemics therefore are tardive rather than explosive (van der Plank 1963). This concept is virtually ignored in all treatments of epidemiology, yet it is extremely important in the epidemiology of perennial diseases of perennial plants such as forest, shade, and fruit trees. White pine blister rust is of particular interest because it is caused by a heteroecious rust and behaved as a compound interest disease over a period of years on both the pine and *Ribes* hosts. The data of Lachmund (1934) also illustrate very well the effect of distance from a point source of inoculum upon both disease severity and infection rate.

In summary, based on available published data on white pine blister rust epidemics in North America, r values of about 0.4-0.6/yr or greater can be expected on sapling to small pole-sized pines growing on high hazard sites and 0.3/yr or less on moderate to low hazard sites. Additional studies should be done in areas of demonstrated low and moderate hazard (van Arsdel 1961) to determine whether the above estimates of r are valid for such sites. These data can be used directly in management plans to estimate potential losses in young stands in the absence of control programs.

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THE EPIDEMIC COURSE OF *CRONARTIUM FLACCIDUM* BLISTER RUST ON THE INTERMEDIATE HOST *VINCETOXICUM HIRUNDINARIA*

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Cronartium flaccidum (Alb. et Schw.) pathogenic agent of two-needled pine blister rust has, as with many other rusts, a cycle which fortunately does not always result at epidemic levels.

In Italy the epidemic wave which struck the pine was mostly restricted to the period between 1960 and 1970 with different incidences (Moriondo 1975). In recent years, and particularly from 1974 to 1975, there have been no significant cases of infection. In the meantime, however, the microorganism has continued to differentiate itself on the intermediate host *Vincetoxicum hirundinaria* Med., even though its development has varied from year to year.

We consider it timely to deepen the epidemiological studies with regard to this organism so as to explain recent observations involving the decrease of the infection. We conducted a study, over the span of 10 years (1976-85), directed to ascertaining the link between climatic factors and the course of infection on the intermediate host.

MATERIALS AND METHODS

The study of epidemic development of *C. flaccidum* on the intermediate host *V. hirundinaria* was carried out in the San Rossore (Pisa) area, along the coast of Tuscany, where *Vincetoxicum* population is abundant. We chose one population under natural covering with a light intensity of 4000 lux.

Observations began in June, continued weekly and terminated in July for a total of 8 weeks. Our observations pertain to the years 1976-85.

The incidence of infection was measured according to a scale based on the percentage of leaf surface infected with telial columns which were measured with a grid mesh of 25 mm^2 (placed on the leaf's surface itself) and accorded median values derived from the observations on three separate leaves: basal, median and apical.

The increase with respect to the preceding values was based on their average.

The germinability of basidiospores on water agar, at 20°C, was measured at the end of July during the final observation of each year.

During the years 1976-85 we analyzed temperature and rainfall data for the months of June and July, calculating a median figure for each 7-day period.

RESULTS

It appears as though the intensity of infection (expressed as the percentage of leaf area infected by the telial columns) at the onset of our observation (first week in June, with observations made between 1976 and 1980) are closely linked to different climatic conditions and above all on extremely varying rainfall levels. In fact, rainfall levels range from 0 to 59 mm and reflect the high geographic and climatic variability of our country (Fig. 1).

Observations made throughout the years appear as though, once the infection process has started, it can undergo pauses in its advance if there is a decrease in rainfall levels. By the same token when sudden variations in rainfall take place, it likewise varies the intensity of infection.

Referring to Table 1, it appears as though the values are low with respect to increase, with the exception of some very high values that correspond to the rise in rainfall levels.

It is interesting to note how, in the years 1980, 1983, 1984, and 1985, the course of infection follows the course of rainfall. In the same years, the greatest increases in the levels of infections correspond to the highest levels of rainfall, or to extremely high values of a survey with respect to a previous survey (Table 1 and Fig. 1, 2).

The years for which the greatest intensity of infection were registered (on final observations) are: 1978, 1981, 1983, 1984, and 1985, with values equal to 59, 55, 52, 57, and 50% respectively. Nevertheless, even within these years there appears a difference in the development of the infection. In fact, during the years 1978 and 1981, the first survey already showed an intensity equal to 40.4 and 39.6% respectively, while during the years 1983, 1984, and 1985, the course was more gradual with infection levels (for the first observations) equal to 10.2, 9.2, and 6.6% respectively.

Based on the average intensity of infection, which was derived from values of eight surveys, the years with the highest intensity were 1978 (56.73%), 1981 (53.7%), 1979 (41.21%), and 1976 (42.11%). For the other years, the average remained less than 40%.

Finally, from Figure 3, one sees how the germinative power of the basidiospores is slightly more than 10% in the years in which temperatures were greater than 20°C with rainfall levels lower than or very near the average.

On the other hand, 1983, 1984, and 1985 show the highest germinative power equal to 71.2, 65.0, and 81.2%, respectively. These years were those which demonstrated, in the last survey in July, the highest rainfall levels equal to 32, 28, and 36 mm with accompanying temperatures of 24, 22, and 26°C.

DISCUSSION AND CONCLUSION

The epidemiological development of *C. flaccidum* on *V. hirundinaria* appears to be directly connected to rainfall. Where monthly levels reach between 30 and 40 mm, the incidence of infection is high.

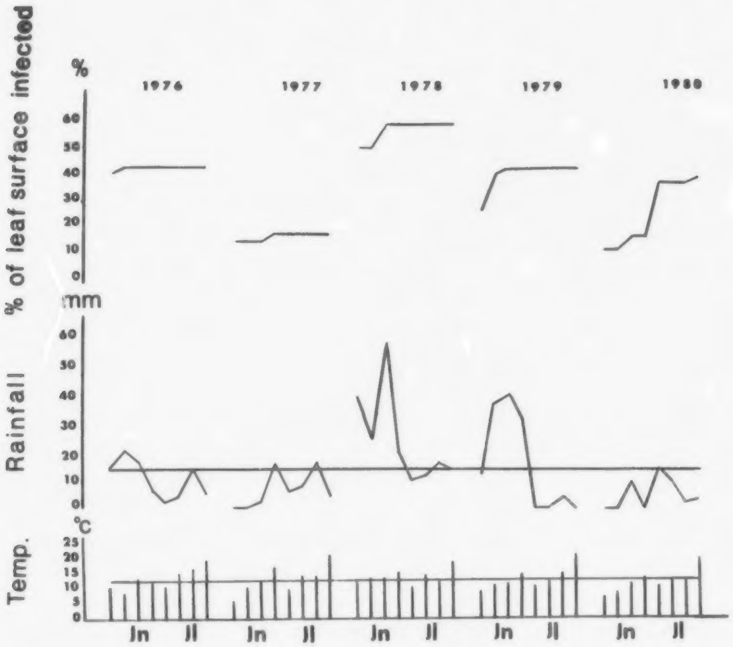

Figure 1. **Behavior of the infection on *V. hirundinaria* in accordance with climatic parameters (1976-80).**

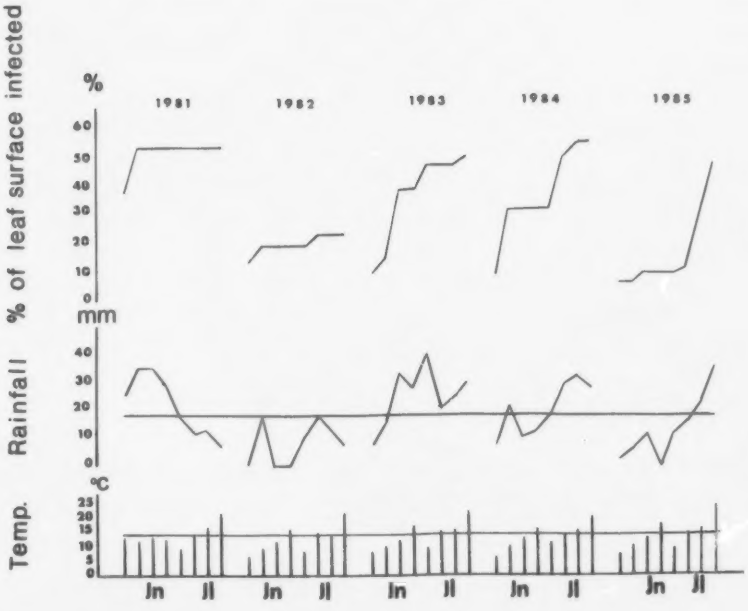

Figure 2. **Behavior of the infection on *V. hirundinaria* in accordance with climatic parameters (1981-85).**

Table 1. Percentage of leaf area infected with telia

	Sample							
Year	I	II	III	IV	V	VI	VII	VIII
1976	*[a]39.7	42.2	42.2	42.2	42.2	42.2	42.2	44.0
	**[b]	2.5	0.0	0.0	0.0	0.0	0.0	1.8
1977	15.2	15.2	15.2	18.0	18.0	18.0	18.0	22.0
		0.0	0.0	2.8	0.0	0.0	0.0	4.0
1978	50.4	50.4	58.6	58.6	58.6	58.6	58.6	60.0
		0.0	8.2	0.0	0.0	0.0	0.0	1.4
1979	27.4	41.2	43.2	43.2	43.2	43.2	43.2	45.1
		13.8	2.0	0.0	0.0	0.0	0.0	1.9
1980	13.8	13.8	18.1	18.1	38.3	38.3	38.3	40.3
		0.0	4.3	0.0	20.2	0.0	0.0	2.0
1981	39.6	55.5	55.5	55.5	55.5	55.5	55.5	57.0
		15.9	0.0	0.0	0.0	0.0	0.0	1.5
1982	14.1	20.0	20.0	20.0	20.0	24.1	24.1	25.0
		5.9	0.0	0.0	0.0	4.1	0.0	1.1
1983	10.2	15.1	40.1	40.1	48.8	48.8	48.8	52.1
		4.9	25.0	0.0	8.7	0.0	0.0	3.3
1984	9.2	33.3	33.3	33.3	33.3	52.6	56.0	57.0
		24.1	0.0	0.0	0.0	19.3	3.4	1.0
1985	6.6	6.6	10.1	10.1	10.1	12.3	30.3	49.2
		0.0	3.5	0.0	0.0	2.2	18.0	18.9

[a] * = Average.

[b] ** = Increase.

It appears obvious that these variables are closely linked. Such is their connection that, in periods where rainfall decreases or remains static for a given length of time, the course of infection stops, only to resume when rainfall levels begin to increase again with respect to the previous period.

Ragazzi (1983) has already demonstrated the role of high rainfall in the differentiation of the telial columns.

Numerous authors have underscored the relation between rainfall and *C. ribicola* Fisch. Spaulding (1922) held that the formation of telial columns, following the reinfection of uredia on *Ribes* spp., is linked to the presence of water on the leaf surface or to a state of atmospheric saturation.

Mielke (1943) asserted that excessive summer rainfall impedes the formation of teliospores.

Riker et al. (1947) have ascertained that to have a good differentiation of telial columns, it is important that the plant of *Ribes* spp. be maintained in a humid environment for 5-12 hours.

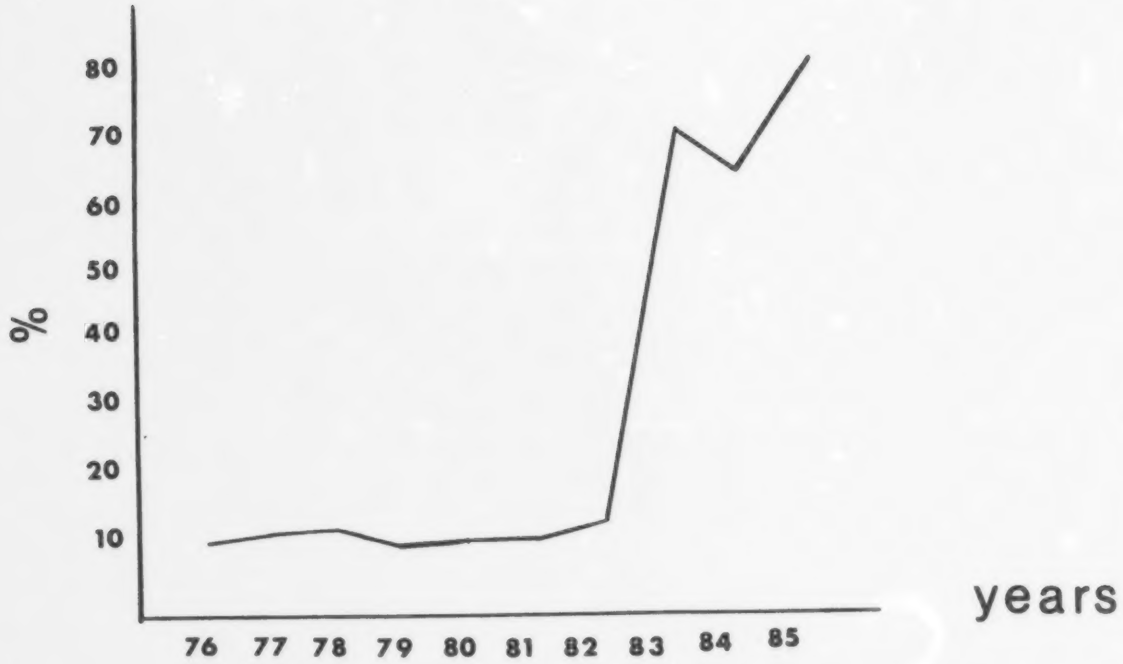

Figure 3. Behavior of germinative power of *Cronartium flaccidum* basidiospores.

Finally, according to Nighswander and Patton (1964) with regard to *Cronartium quercuum* (Berk.) Miyabe ex Shirai, the number of telial columns increases with the rise and duration of humid periods.

The overall surveys for 1978 revealed that year to be the one with the greatest number of infections. Such was the case even though higher peaks were registered in other years. Analyzing the temperature and rainfall data for each year, one finds that the only variable that stands out is the course of temperature (which is always higher than the average); consequently, under the same rainfall conditions, the temperature is to be considered the only significant variable.

Past experience has already shown that temperatures between 15 and 25°C are highly favorable to the formation of telial columns (Ragazzi 1983).

With regard to *C. ribicola*, we refer to a number of authors who have reported similar findings.

According to Hirt (1935), fertile telial columns form within a temperature range of 0-21°C.

Lloyd (1958) asserts that temperature differences vary from 1 to 20°C with 16°C being optimum. Nighttime temperatures of 20°C can impede the formation of telial columns while daytime temperatures of 35°C can completely obstruct them, notwithstanding the eventual favorable conditions which follow at night. Telial columns that form at 20-24°C do not sporulate.

Harvey (1972) affirms that the production of telial columns increases if, during the period of their formation, the plant (*Ribes* spp.) undergoes a period of low temperatures.

Spaulding and Rathbun-Gravatt's (1925) research agrees with the above-mentioned authors.

Riker et al. (1947) found that the development of telial columns is abundant at 16°C, good at 20°C, sporadic at 24°C, and nil at 28°C.

Van Arsdel (1954) and van Arsdel et al. (1953) demonstrated that telial columns that form at 20°C are irregular with regard to sporulation, while those that form at 24°C are sterile.

Van Arsdel et al. (1956) conducted a thorough study on the influence of temperature on the formation of teliospores, determining that temperature influences teliospore vitality. Indeed, temperatures ranging from 24 to 28°C practically nullify sporulation power. Teliospores that form over a period of 3 weeks with temperatures ranging from 15 to 25°C become sterile even though 1 week with temperatures less than 20°C allows for fertile teliospores. Teliospores from other origins revealed good fertility when produced during periods with daytime temperatures between 28 and 30°C and nighttime measurements between 5 and 15°C. Temperatures between 28 and 32°C (over a period of 3 days) were enough to render teliospores, originating from a third location, sterile.

From these results one can deduce that the intensity of infection, even though varied over the years, reached values (from 6.5 to 59%) that could lead one to presume an equally high incidence of infection on the pine. In reality, however, this has not been verified. On the contrary, there has been a decrease in the intensity of infection since 1976-77. Such may be due to the course of extremely low germinative power of the basidiospores (around 10%) during the period between 1976 and 1982.

In 1983, and in the following years (1984 and 1985), germinative power abruptly increased in connection with a period where high levels of rainfall ran parallel to higher-than-average temperatures. Under these conditions there was still not a corresponding increase of infection on the pine. Therefore, other factors must be in operation or acting on the basidiospore that further lower its germinative power or something occurring with regard to pine needles.

Some considerations to be made at the close of the study:

- the conditioning factor for *C. flaccidum* infection on *V. hirundinaria* (telial columns) is rainfall;
- under the same rainfall conditions, the temperature is to be considered the only significant variable;
- cases of high intensity infection on *V. hirundinaria* did not correspond to high germinative power of the basidiospores;
- when the germinative power of the basidiospores demonstrates itself to be high (greater than 65%), there is, nevertheless, no corresponding increase of infection on the pine;

- the decrease in infection on the pine beginning in 1976-77 could be the result of a low germinative power of the basidiospores;

- the low germinative power of the basidiospores, however, cannot be considered the only determining factor in an almost nonexistent increase of infection on the pine;

- it seems evident that it is necessary to investigate the role of the climatic variables which occur during the period of formation (day and night) of telial columns.

SUMMARY

Over the span of 10 years (1976-85), a study directed to ascertaining the link between climatic factors and the course of *Cronartium flaccidum* infection on the intermediate host *Vincetoxicum hirundinaria* was carried out. The research took place in San Rossore (Pisa) at sea level. The observations began in June and continued weekly until July for a total of 8 weeks each year.

The results obtained were:

- the factor conditioning the blister rust infection on *V. hirundinaria* (telial columns) is the rainfall;

- under the same rainfall conditions, the temperature is to be considered the only significant variable;

- the decrease of infection on pine beginning in 1977-78 could be the result of a low germinative power of basidiospores.

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THE USE OF MOLECULAR MARKERS IN STUDYING PONDEROSA PINE–*PERIDERMIUM HARKNESSII* INTERACTIONS

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Over the past three decades, forest geneticists and pathologists have made considerable progress towards the development of screening systems involving resistance of loblolly (*Pinus taeda* L.) and slash (*P. elliotii* Engelm.) pines to fusiform rust (*Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*), and sugar (*P. lambertiana* Dougl.) and western white (*P. monticola* Dougl.) pines to white pine blister rust (*C. ribicola* J.C. Fischer ex Rabh.) (McDonald and Hoff 1990; Powers 1990). Despite these successes, little progress has been made in the description of the mechanisms and the isolation of genetic components that determine the outcome of the host-pathogen interactions. Recently, molecular biologists have attempted to identify and characterize resistance genes (Riggleman et al. 1985; Schweizer et al. 1989). The initial optimism associated with this approach in plant pathology has given way to the realism that isolating genes responsible for resistance to biotic factors is more complicated than isolating genes responsible for resistance to abiotic factors such as herbicide resistance (Shah et al. 1986). Moreover, the random molecular examination of the host genome alone can be inefficient in locating genes responsible for host-pathogen interactions.

An integrated approach, utilizing the principles and techniques of genetics, plant pathology and molecular biology, in gene-for-gene modeling of host-pathogen interactions may prove more systematic in the identification and isolation of genes responsible for these interactions. Geneticists and pathologists can provide basic information that will increase the probability of successfully using molecular techniques to identify genes responsible for host-pathogen interactions. Such information includes the definition of the breeding populations, description of the host reaction to infection and estimation of the number and type of genes involved in a specific host-pathogen interaction. The use of molecular markers, i.e., genetically controlled phenotypic differences among individual trees or fungal isolates, provides an opportunity to integrate these three disciplines.

The objective of this paper is to describe our approach in the application of molecular markers in studying host-pathogen interactions. Specifically, the use of molecular markers will be discussed in the context of: 1) description of genetic variability in the host and pathogen, 2) characterization of gene action responsible for host-pathogen interactions, and 3) identification and isolation of genes responsible for a specific host-pathogen interaction. The ponderosa pine (*Pinus ponderosa* Dougl. ex Laws var. *scopulorum*)-western gall rust (*Peridermium harknessii* J.P. Moore) pathosystem is presented as a model because of the historical information available for ponderosa pine and because of the unique life cycle of *P. harknessii*.

GENETIC VARIABILITY

The description and characterization of the amount of genetic variability in host and pathogen populations is a critical component in the study of host-pathogen interactions. Molecular techniques can be used to complement traditional methods to provide additional tools for the quantification of genetic variability. Isozyme techniques (Tuskan and Walla 1989) and restriction fragment length polymorphisms (RFLP) procedures (Bernatzky and Tanksley 1986; Helentjaris et al. 1985) provide reliable molecular markers that have been used to quantify genetic variability in pines and fungi (Bonde et al. 1984; Cook et al. 1989; Karalamangala and Nickrent 1989; Wagner et al. 1987).

Pathogen Variability

With *P. harknessii*, isozyme data indicate that isolates in the north-central United States are homozygous and that geographic populations of groups of isolates are heterogeneous (Tuskan and Walla 1989). Furthermore, Tuskan (1989 unpublished data) has identified 24 pathogen electrophoretic types distributed among 13 sampled populations based on a composite of five polymorphic loci (Table 1). Some populations had as few as 1 electrophoretic type, whereas others had as many as 10. Nei's (1972) coefficient of genetic distance among populations, through the use of cluster analysis (SAS Institute Inc. 1985), suggests that two main clusters that correspond to host origins east or west of North Dakota can be identified within the pathogen population (Fig. 1).

Information of this type has implications in the study of ponderosa pine-*P. harknessii* interactions. Isozyme variability among isolates indicates there is genetic variability in the pathogen, which suggests variability in genes controlling host-pathogen interactions also may exist. As a practical consequence, several *P. harknessii* isolates from each cluster would have to be used to adequately characterize ponderosa pine-*P. harknessii* interactions. Moreover, the homozygosity revealed by isozyme analysis implies outcrossing is rare. If this is true, the structure of gene-for-gene models of host-pathogen interactions can be simplified.

RFLPs can be used to obtain additional genetic markers. DNA probes corresponding to low copy number sequences in *P. harknessii* have been isolated, and one probe, PHA1, in combination with Hind III digestion of fungal DNA has revealed polymorphisms among electrophoretic types, as well as polymorphisms within electrophoretic type A (Wang, 1989 unpublished data). The existence of these markers increases the probability of finding markers linked to genes responsible for specific host-pathogen interactions.

Host Variability

Isozyme and RFLP analyses can be applied to host material to identify markers used in quantifying genetic variability. In ponderosa pine, approximately 23 isozyme loci have been identified that can be used as molecular markers (O'Malley et al. 1979). Variability among individuals in molecular markers and disease resistance can be generated through the use of mating designs applied to genetically diverse host materials. For example, based on reports from Pennsylvania (Merrill et al. 1986), Michigan

Table 1. A summary of the electrophoretic characterization of *Peridermium harknessii* isolates from 13 geographic populations in the north-central United States

Population	Electrophoretic type[a]	Occurrence (%)	Number of isolates[b]
Dominated by Type A			
Slope Co., ND	A,G,H,J,R	71.4, 14.2, 4.8, 4.8, 4.8	22
Sloper Co., ND	A,H	90.5, 9.5	21
Oliver Co., ND	A,B,G,H	50.0, 16.7, 16.7, 16.7	10
McHenry Co., ND	A,B,F,G,H,O	73.2, 7.7, 3.8, 7.7, 3.8, 3.8	26
Cass Co., NE	A	100.0	25+
Adams Co., NE	A,B	90.9, 9.1	25+
Not dominated by Type A			
Pembina Co., ND	A,B,I,K,L M,P,Q,R,S	26.6, 6.7, 6.7, 6.7, 6.7 6.7, 6.7, 6.7, 6.7, 20.0	20
Pembina Co., ND	D,L,S,T,U V,W	10.0, 20.0, 20.0, 5.0, 30.0, 10.0, 5.0	22
Beltrami Co., MN	L,Q,R,S	20.0, 20.0, 20.0, 40.0	10
Traill Co., ND	D,E,M,W	20.0, 20.0, 20.0, 40.0	5
Stutsman Co., ND	C,D,W,X	12.5, 62.5, 12.5, 12.5	11
McIntosh Co., ND	G	100.0	2
Bowman Co., ND	G,N	50.0, 50.0	2

[a] Based on a composite of the electromorphs of the polymorphic isozymes ACP (3.1.3.2), CAT (1.11.1.6), EST (3.1.1.1), GOT (2.6.1.1), and PGM2 (2.7.1.5) (Tuskan and Walla 1989).

[b] The number of electrophoretic types per population is not correlated to the number of samples per population.

(Thomas et al. 1984), Nebraska, and Missouri (Peterson, 1987 personal communication), provenances within a ponderosa pine seed source study have been ranked and selected based on field reactions to *P. harknessii*. Eighteen highly susceptible and 22 phenotypically resistant female parents from within putatively susceptible and resistant seed sources, respectively, have been crossed with two unrelated highly susceptible and two unrelated phenotypically resistant male parents (Table 2). Progeny segregating for genes controlling host-pathogen interactions can be inoculated with geographically diverse inoculum sources to obtain estimates of additive and dominance gene action (i.e., horizontal or vertical resistance, respectively). Quantifying genetic variability in the host progeny will allow an estimate of the type(s) of gene action involved in specific host-pathogen interactions, as well as an estimate of the amount of linkage between molecular markers and resistance genes.

CHARACTERIZATION OF GENE ACTION

Gene-for-gene models of host-pathogen interactions, as discussed by Loegering (1984) and Jenns and Leonard (1985), can be fit to the progeny inoculation data to further characterize gene action. Models involving one, two, and three dominance genes can be tested for goodness-of-fit by using the chi-square test. In a one-gene model with dominance gene action there are two alternative combinations of host-pathogen genes with four interactions each (Table 3). The difference among combinations relates

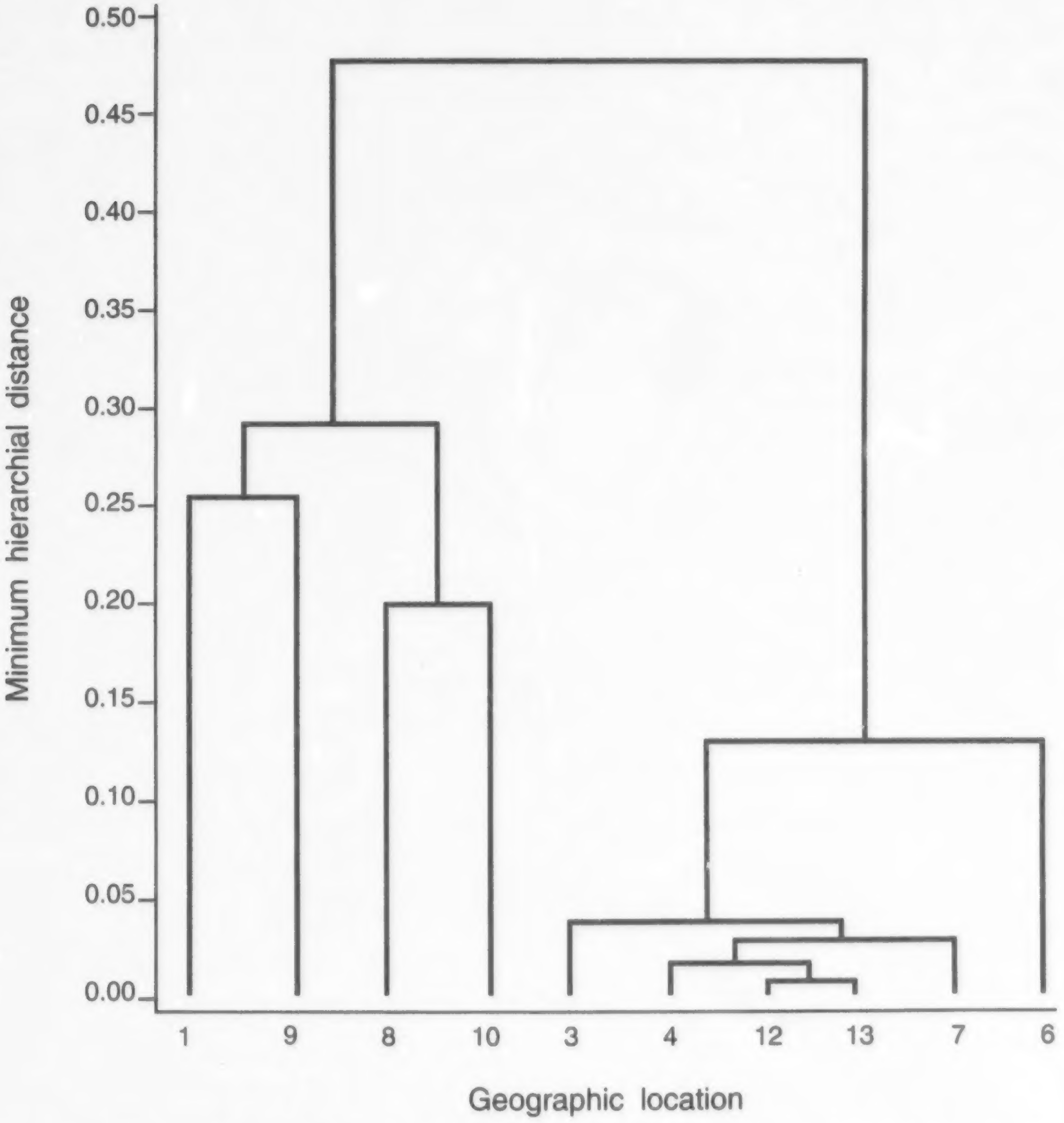

Figure 1. Geographic separation of *Peridermium harknessii* isolates collected from 13 locations in the north-central United States.

Table 2. Summary of the crosses and genetic information obtained from a tester mating design utilizing seed sources in a 20-year-old ponderosa pine provenance study

Female parent	N	Male parent	
		Resistant	Susceptible
Resistant	18	X	X
Susceptible	22	X	X

Source of variation	Estimates
Males	Additive genetic variance
Females	Additive genetic variance
Males · Females	Dominance genetic variance

Table 3. An example of possible gene-for-gene interactions between host and pathogen with a one-gene model

	Virulence genes[a]	
Resistance	V_1	v_1
Dominant		
3 R_1–[b]	+[c]	–
1 r_1r_1	+	+
Recessive		
3 R_1–	+	+
1 r_1r_1	+	–

[a] V_1 indicates virulent allele and v_1 indicates avirulent allele.

[b] – indicates either a recessive (r) or dominant (R) resistant allele.

[c] + indicates a susceptible or compatible reaction, assuming virulence overcomes resistance, and – indicates a resistant or incompatible reaction.

to differences in the expression of resistance; i.e., differences between a recessive or dominant trait. The particular interaction within each combination, + or –, depends upon the host and pathogen genotypes and the particular trait being examined. With ponderosa pine-*P. harknessii*, the host-pathogen interaction could be evaluated based on the presence or absence of traits such as 1) spore germination on the host surface, 2) haustoria or intercellular mycelium in host cells, 3) tissue pigmentation, or 4) stem gall formation (Lundquist et al. 1990). Each of these traits may be affected by genes that operate independently or in tandem with other traits. Additive gene action can be incorporated into the gene-for-gene models presented in Table 3 and tested using a chi-square test (Table 4). Traits such as the size of pigmented area, rate of symptom development, size of stem gall, or relative reduction in seedling growth may be expected to be under the control of both additive and dominance genes. The chi-square test will validate the estimates of additive and dominance gene action from the mating design and give an indication of the number of genes involved in the host-pathogen interaction.

Estimates of the number of genes controlling a particular interaction can be used during linkage analysis involving molecular markers and the occurrence of a postinoculation reaction in the host. If gene-for-gene models indicate that one, two, or three genes control a specific host reaction, then a minimum of one, two, or three markers will have to be found to locate genes responsible for that reaction, assuming no linkage among resistance genes. Based on evidence from molecular markers in *P. harknessii*, it is likely that alternative pathogen genotypes are present in inoculum populations from geographically diverse sources. It is also likely that varied host-pathogen combinations will yield alternate post-inoculation reactions that could provide the basis for mRNA selection through competitive cDNA hybridization techniques.

Table 4. Estimates of disease severity given a gene-for-gene model with one set of dominance genes in the host (R_1, r_1) and pathogen (V_1, v_1) associated with resistance and virulence, respectively, and a set of additive genes in the host (R_A) and pathogen (V_A) which modify the dominance gene interactions

Host genotype	Pathogen genotype					
	v_1v_1,OV_A	v_1v_1,V_A	v_1v_1,2V_A	V_1V_1,OV_A	V_1V_1,1V_A	V_1V_1,2V_A
R_1–, OR_A	0	0	0	1	2	3
R_1–, 1R_A	0	0	0	0	1	2
R_1–, 2R_A	0	0	0	0	0	1
r_1r_1, OR_A	1	2	3	1	2	3
r_1r_1, 1R_A	1	2	3	1	2	3
r_1r_1, 2R_A	1	2	3	1	2	3

Assumptions:
1) Dominance and additive genes are independent.
2) Additive genes interact only with additive genes to modify a susceptible reaction.
3) Resistance and virulence are dominant and virulence overcomes resistance.
4) Disease severity = (No. of R_{1-}, V_1V_1 matches) + (No. of V_A genes – No. of R_A genes: a R_1–, V_1V_1 match) + (No. of r_1r_1 genotypes) + (No. of V_A genes: an r_1r_1 genotype), where 0 indicates a resistant or incompatible reaction and 3 indicates a severe susceptible or compatible reaction.

ISOLATION OF GENES RESPONSIBLE FOR HOST-PATHOGEN INTERACTIONS

Upon pathogen attack, a vast array of molecular responses is initiated in the host (Lamb et al. 1989). These responses may occur in susceptible and resistant genotypes, but the responses vary between susceptible and resistant individuals in time, product, or site (Bell et al. 1986). The response may be controlled by constitutively expressed genes present in the resistant genotype. Competitive cDNA hybridization techniques can differentially select unique mRNA sequences found in one genotype. These techniques and linkage information can be used to isolate genes responsible for resistant interactions between ponderosa pine and *P. harknessii* (Fig. 2). Progeny from a single cross which are segregating for a post-inoculation reaction can be used to isolate RNA messages after inoculation. The mRNA from progeny displaying a compatible (susceptible) reaction are used to create cDNA hybridization selection with mRNA from progeny displaying an incompatible (resistant) reaction. The mRNA from resistant progeny that does not hybridize may contain sequences responsible for incompatible reactions in the host. The cDNA clones can then be created from these mRNA's and screened with mRNA from the compatible genotype, further selecting potential clones carrying resistance sequences in the host cDNA library (Rigglemen et al. 1985; Schweizer et al. 1989). Genetic analysis of these clones using RFLPs and other molecular markers linked to resistance can further identify clones with putative resistance genes. These cDNA clones can then be analyzed to determine the nature of the host DNA sequences.

SUMMARY

Molecular markers can be used to describe genetic variability in host and pathogen populations, thus allowing inoculations involving a broad genetic base and assuring alternate post-inoculation reactions. Host-pathogen interactions leading to resistance may occur through several mechanisms that are independently inherited yet function in concert to express an incompatible (resistant) reaction. Understanding the nature of this reaction will allow the identification of traits that are controlled or modified by dominance and additive genes. Competitive cDNA hybridization techniques can be used alone to isolate such genes, but such techniques are inefficient in view of the numerous genes that may vary among progeny segregating for the control of the host-pathogen interaction. Linkage of molecular markers to simply inherited traits will improve the efficiency of competitive cDNA hybridization techniques for the isolation of such genes. Moreover, using the principles of gene-for-gene interactions, inoculation data may indicate the number of molecular markers needed to identify genes responsible for the host-pathogen interaction.

The apparent difficulties in describing, and therefore isolating, resistance genes in a host-pathogen system may offer unrealized advantages. Genetic information from either the host or the pathogen, in view of gene-for-gene models of host-pathogen interactions, can be used to improve the probability of isolating genes controlling the host-pathogen interaction. An integrated combination of technical approaches and expertise of geneticists, plant pathologists and molecular biologists will provide the basis for the description of such genes.

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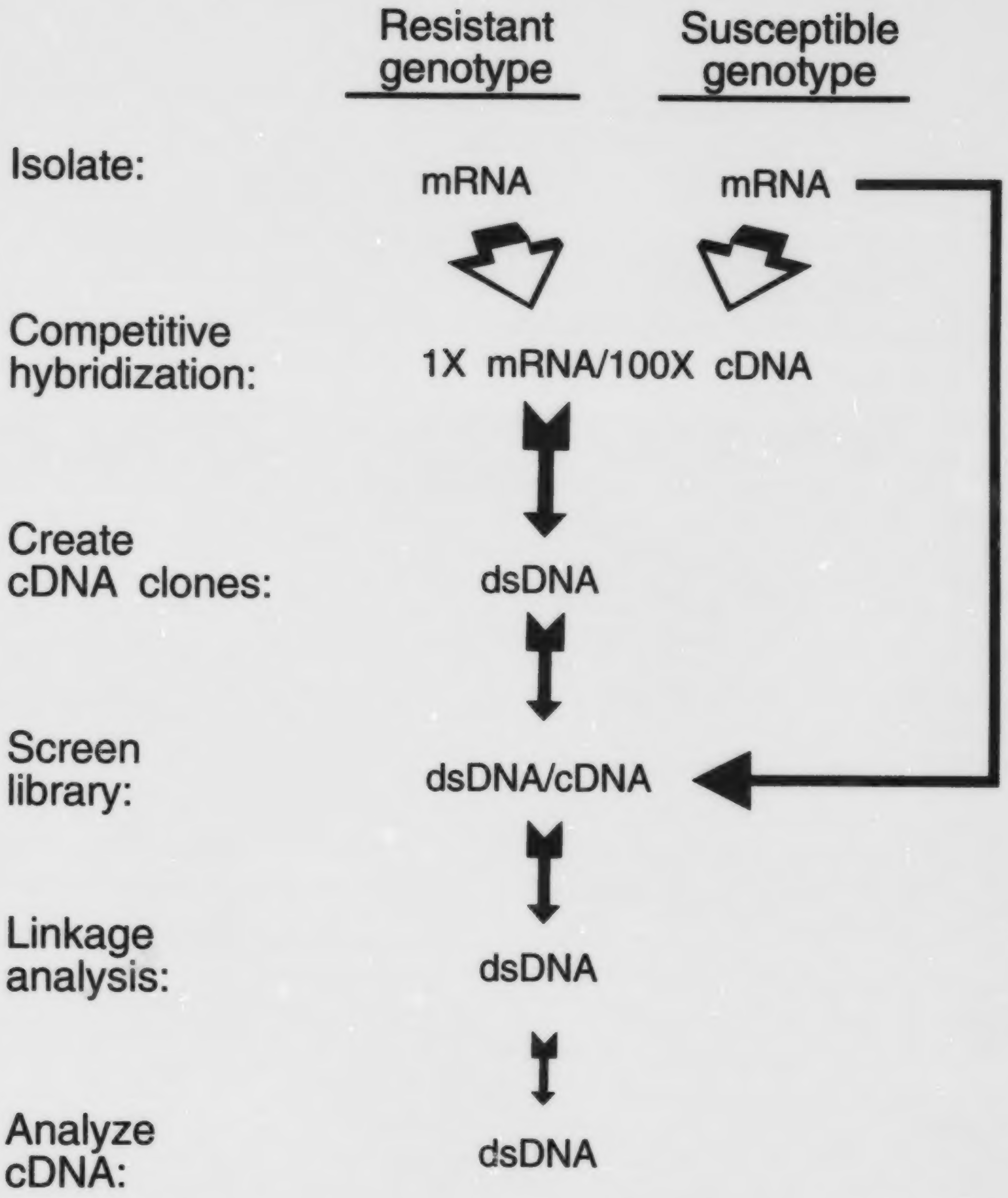
Resistant genotype
Susceptible genotype
Isolate:
mRNA
mRNA
Competitive hybridization:
1X mRNA/100X cDNA
Create cDNA clones:
dsDNA
Screen library:
dsDNA/cDNA
Linkage analysis:
dsDNA
Analyze cDNA:
dsDNA

Figure 2. A schematic representation of a competitive cDNA hybridization technique for the isolation of genes responsible for an incompatible reaction in a host-pathogen interaction.

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THE EXPRESSION OF RESISTANCE IN THE LODGEPOLE PINE–WESTERN GALL RUST PATHOSYSTEM[1]

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ABSTRACT

The effects of random placement of spores on hosts on the variation in number of infections per tree are explored. It is shown that this source of variation can be substantial, and that it can be modelled by the Poisson distribution. In addition, two possible ways in which resistance mechanisms might interact are described.

SOURCES OF VARIATION IN NUMBER OF INFECTIONS PER TREE

One of the basic tenets of population genetics is the view that phenotype (that is, the way that an organism appears) is the result of the genetic makeup of that organism and the environment to which it has been exposed over time. Thus $P = G + E + G \times E$, where P is the total phenotypic variance, G the genetic variance, and E the environmental variance. That basic relationship, in variously expanded forms, represents one of the basic paradigms of population genetics. It expresses our belief about the nature of things; it has not to my knowledge ever been proven. Analysis of variance has provided the ideal tool for dissecting the world according to this paradigm. However, an ANOVA always has an error term, which means that part of the total variation is not accounted for. Now normally it seems that the unaccounted variation can be wholly attributed to one or more of the sources of variation that were not fully controlled in the experiment. But this, of course, is a belief, not a proof. There is always the danger that a particular source of variation is overlooked and included with the error term.

In studies of the inheritance of disease resistance, the phenotype of interest is disease severity. For many diseases, that phenotype must of necessity be measured by a set of arbitrarily defined categories. Stem rusts of pine, and particularly western gall rust (*Endocronartium harknessii* (J.P. Moore) Hirat.) on lodgepole pine (*Pinus contorta* Dougl.), allow an actual count of the number of infections per tree, and therefore represent a particularly good opportunity for studies of the inheritance of disease resistance. Thus the phenotypic character of interest, for the discussion of disease severity and the inheritance of resistance in pine rusts, is the number of infections per tree.

The number of infections that appear on a tree is determined by a large number of factors that can be divided into three groups and their interactions, namely, the host genetic factors, the environmental factors, and the inoculum factors. The previous sentence is a restatement of the paradigm described above. It says that if we were able to control all these factors, or determine their effect, then we would be able to predict the number of infections per tree precisely for any set of conditions. Another

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way of saying this is that, if we were to repeat an inoculation experiment under identical conditions, we would get an identical result. Today I want to examine the meaning of "identical conditions" and "identical results" more closely.

The methodology of most inoculation experiments aims to control the amount and quality of inoculum in such a way that each individual receives the same dosage. The desired dosage normally consists of fixed number of spores that are randomly deposited on that individual. The actual infection process occurs through a microscopic infection court. Each individual has many such courts and, in the normal case, only a few of these become infected. Whether a particular court becomes infected will depend on whether a viable spore has landed in a way, and under a set of environmental conditions, that will ensure germination and penetration.

Under such conditions, each infection court can be thought of as having a small probability of becoming infected. The expected number of infections on a tree is the sum of the probabilities of infection of all the infection courts that have existed in the life of that tree. I propose to call that sum the "infection rate", and suggest that the statement above should be reformulated to say that, if an inoculation experiment is repeated under identical conditions, then the same infection rate should result. By "identical conditions" I mean the same dosage deposited in the same random fashion under the same environmental conditions on the same set of hosts.

If the number of infections that will appear on a tree is determined by the outcome of many microscopic events, each with a characteristic but small probability of success (the magnitude of which is determined by host genetics, the inoculum dosage and the environmental conditions), then the actual number that will appear on the tree is not fixed. Rather there will be a distinct probability that a tree with a given infection rate will have 0, 1, 2, or some other whole number of infections. Imagine, for instance, a simple case in which a tree has 1000 infection courts, each with a probability of 0.0015 of becoming infected. In such a case, the infection rate is 1.5 infections per tree; and the probability of 0, 1, 2, 3, or >3 infections is given by the binomial probabilities for that number of successes when $N = 1000$ and $p = 0.0015$. These are 0.2229, 0.3348, 0.2512, 0.1256, and 0.0655.

Now, we do not know the number nor the precise location of infection courts, nor the likelihood of their infection. If we did, no further experimental work would be necessary, because we would then have a precise definition of the susceptibility of that tree. However, if we are willing to accept that the number of courts is large, and that the probability of infection of each court, though variable, is always small; then the Poisson distribution approximates the probability of having some discrete number of infections on a tree with a given infection rate. For an infection rate of 1.5 infections per tree (the above example), the Poisson predicts probabilities of 0.2231, 0.3347, 0.2510, 0.1255, and 0.0657 for 0, 1, 2, 3, and >3 infections per tree. The Poisson prediction is based on the infection rate only, and assumes that the number of infections is the outcome of a large number of independent events, each with a very low probability of success.

It follows from these arguments that there is a source of variation in number of infections per tree that is neither genetic nor environmental in the normal sense of these terms. This variation arises because the number of infections on a tree is the outcome of many individual events (the possible penetration of individual infection courts), each of which has a low probability of success.

The variance of a Poisson distribution is equal to its mean. Hence the source of variation described here can be rather large compared to other sources, especially when the number of infections is low. Elsewhere in these proceedings (Kojwang and van der Kamp 1990), we describe an experiment in which 16 pine clones produced by grafting were inoculated by four single-gall spore sources. The measure of infection was number of galls per shoot. In that experiment, the average variance over mean ratio for the 47 spore-by-clone combinations that were infected was 0.985. This suggests that the randomness introduced by the random placement of spores within individual shoots accounted for essentially the whole error sum of squares; and hence that other sources of experimental error, such as variation in the number of spores per shoot or their quality, or variation in experimental conditions related to temperature or moisture, were insignificant.

The view of the infection process and the resulting number of infections per tree (or some other unit of host tissue) proposed here has many implications. For instance, it suggests that the number of infections that appear on a tree in the field is only a rough indication of the infection rate of that tree (or its susceptibility). It also quantifies one of the reasons for escapes, that is, trees that we would expect to be infected under a particular set of conditions, given their genetically determined susceptibility, but that somehow escape infection. At the same time it suggests that the notion of escapes should be extended to overinfection, because we would also expect to find some trees that have many more infections than would be predicted on the basis of their relative susceptibility. Furthermore, the view proposed here has implications for experimental design, because it allows the estimation of an unavoidable source of variation and hence allows a calculation of the minimum number of replications that will be necessary to demonstrate certain effects, even when all other experimental conditions are fully controlled. The example cited above suggests that when the expected number of infections per unit of tissue is low, the source of variation attributable to the random placement of spores is large, relative to other sources. Also, selection of resistant trees should clearly be done in situations where the average number of infections per tree in the stand is very large. If that number is low, the randomness attributable to the phenomenon described here will make selection largely meaningless. Finally, the concepts proposed here allow one to bridge the gap between a continuously varying infection rate and a discrete number of infections per tree. I used to be puzzled by such hypothetical questions as the following: If, under a certain set of uniform conditions, each tree in a clone has one infection, what will be the number of infections per tree if the number of spores is reduced by 50%? The answer I would now give is first, that I would not expect an experimental outcome in which each individual has exactly one infection and, second, that the infection rate will be 0.5, while the actual number of infections that will appear will follow a Poisson distribution with a mean of 0.5.

POSSIBLE INTERACTIONS OF RESISTANCE MECHANISMS

A second basic tenet of population genetics is based on the Central Limit Theorem, and holds that if the magnitude of a certain characteristic is determined by many minor (i.e., essentially undetectable) genes acting in an additive fashion, then the distribution of the magnitude of that character will approach normality. Thus, Fleming and Person (1982) and Carson (1987) propose that if susceptibility to a disease is governed by many minor genes acting in an additive fashion, then disease severity will be inversely proportional to the number of minor resistance genes, and disease severity will be normally distributed. These authors then go on to examine various theoretical interactions between resistance and aggressiveness genes. My purpose today is to examine the assertion that disease severity will be inversely proportional to the number of minor resistance genes for the case of western gall rust.

The infection process of western gall rust is a long one. The fungus must penetrate the cuticle and epidermis, and then grow through the cortex and the newly formed phloem to the cambium. Here it stimulates the cambium to produce a gall. I assume that gall formation is the indication of successful infection. I have never seen spore production without gall formation, and all galls produce spores at least once. Various resistance mechanisms have been postulated or described (Allen et al. 1990), and these occur at various points along the infection pathway, involving all the tissues that are invaded. Some of these, such as periderm formation in the cortex, serve to eliminate some, but not all, of the penetrations.

Blenis and Pinnell (1988) have shown that virtually all seedlings, whether derived from uninfected or heavily infected parents, can be infected if the inoculum load is heavy enough. This corresponds to many field observations, which suggests that as conditions for the disease become more favorable, the percentage of trees infected increases to nearly 100%. This, in turn, suggests that none of the common resistance mechanisms provide absolute resistance against (all races of) the rust, because, if some did, some proportion of trees would remain uninfected under all conditions. Rather, all the common resistance mechanisms appear to reduce the number of successful penetrations by a greater or lesser proportion. Thus, the incomplete, rate-reducing type of resistance mechanism, such as periderm formation in the cortex, appears to be the common type of resistance mechanism operating in lodgepole pine against gall rust.

Figure 1 shows in a diagrammatic fashion how resistance mechanisms might act in parallel and in series. In the former case (Fig. 1A), each mechanism reduces the number of successful penetrations by a fixed amount, independent of whether the other resistance mechanisms are present on that host or not. This is an arrangement that results in the view presented by Fleming and Person (1982) and Carson (1988), and it represents the normal assumption about the relationship between the number of minor resistance genes and disease severity.

If resistance mechanisms act in series (Fig. 1B), as seems likely in the case of western gall rust on pine, a different relationship results. Now, each mechanism reduces the number of successful penetrations by a particular proportion. Different resistance mechanisms interact in a multiplicative, rather than an additive, fashion. As a hypothetical example, one might imagine two resistance mechanisms, one involving the epidermis and the other the cortex, such that either, if present in a tree, would reduce the number of infections by 40%. Now, if a tree carrying neither of these mechanisms had an infection rate of 20 galls per tree, it would have an infection rate of $20 \times (1 - 0.4) = 12$ galls per tree with either one of these mechanisms present, and a rate of $20 \times (1 - 0.4) \times (1 - 0.4) = 7.2$ with both mechanisms present. If resistance mechanisms act in series, the logarithm of the infection rate is proportional to the number of resistance genes.

I present these reflections to call attention to the fact that the common and apparently unchallenged assumption relating to additive resistance, namely, that the susceptibility of a tree as measured by its infection rate (which, in turn, can be estimated more or less precisely from the number of galls that appear on that tree) is directly proportional to the number of minor susceptibility genes (or inversely proportional to the number of minor resistance genes), is not the only biologically reasonable assumption. In fact, a multiplicative relationship may be more likely in the case of the pine-gall rust pathosystem.

One consequence of a multiplicative relationship is that, in a population of trees in which the number of minor resistance genes is normally distributed, most trees will appear to be quite resistant, and a few very susceptible. This can be demonstrated for a simplified case as follows. Imagine that there are 10 resistance mechanisms, each reducing the number of infections by 50%, and each conditioned by a single, independently segregating resistance gene. If the number of such genes is normally distributed, we would expect most trees to have a near average number of genes, and a few trees to have either many or very few. If a tree without any of these mechanisms had an infection rate of 25 galls per tree (under

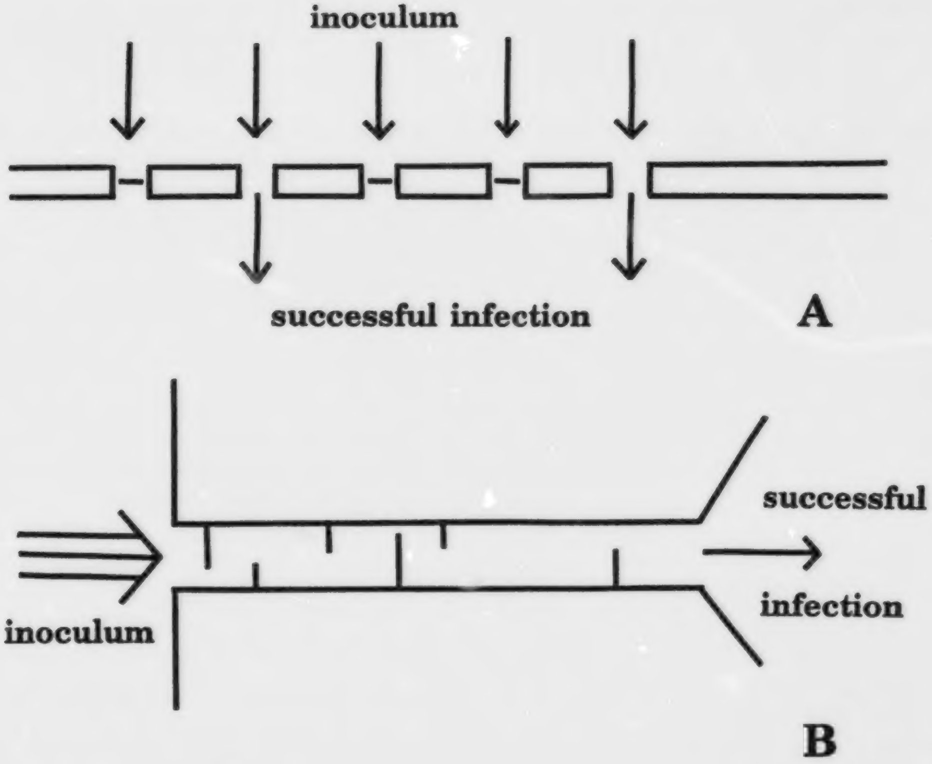
inoculum
successful infection
A
successful
infection
inoculum
B

Figure 1. Representation of the relationship between separate resistance mechanisms. **A)** a parallel arrangement resulting in an additive relationship. The blocked openings represent effective resistance mechanisms; the open ones infection pathways. **B)** a series arrangement resulting in a multiplicative relationship. The infection pathway is partially blocked by resistance mechanisms at various points. The efficacy of these mechanisms is represented by the size of the blockage.

a standard set of environmental and dosage conditions), then trees with 0, 1, 2, ..., 10 resistance mechanisms would have infection rates of 25, 12.5, 6.25, 3.125, 1.562, 0.781, 0.391, 0.195, 0.098, 0.049, and 0.024 infections per tree. Under these conditions, the trees in the six most resistant classes would remain mostly free of infection, the trees in the next three classes would have a few infections, and the few remaining trees would have a very large number of infections. This follows closely the pattern normally observed in the field. Most trees are either uninfected or have a very few infections, while some trees are heavily infected. Thus, the distribution of disease severity predicted by a multiplicative interaction of resistance mechanisms is in accord with field observations. On the other hand, the additive model predicts that most trees should have an intermediate number of infections.

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INFECTION OF LODGEPOLE PINE CLONES BY SINGLE-GALL ISOLATES OF WESTERN GALL RUST[1, 2]

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ABSTRACT

Sixteen clones of lodgepole pine produced by grafting were inoculated by four single-gall spore sources of western gall rust. Clones and spore sources varied in resistance and pathogenicity. A significant interaction was also detected and attributed to the behavior of four clones that showed a pattern of infection different from the other clones. Individual rust isolates caused infection on 62 to 75% of the clones tested. Single major resistance and virulence genes may play a role in this pathosystem, but polygenic resistance expressed as variation in infection rates appears to predominate.

INTRODUCTION

Western gall rust (*Endocronartium harknessii* (J.P. Moore) Hirat.) is the most common stem rust of lodgepole pine (*Pinus contorta* Dougl.) in Canada (Ziller 1974). It is a native disease and occurs throughout the range of lodgepole and jack (*P. banksiana* Lamb.) pine except perhaps at their extreme northern limits. The rust has a simple life cycle. Spores produced on discrete woody galls in the spring and early summer infect newly emerging pine shoots. Spermogonia are only rarely produced. Hence, if karyogamy and meiosis occur at spore germination (Hiratsuka et al. 1966), the former probably involves the fusion of two identical nuclei. Alternatively, nuclear divisions at germination may be strictly mitotic (Epstein and Buurlage 1988; Kojwang 1989). Isozyme analysis by starch gel electrophoresis suggests that *E. harknessii* is homozygous (Tuskan and Walla 1989). Thus the offspring of a single infection will normally be genetically identical to that infection, and the rust probably consists of distinct races.

The western gall rust pathosystem has many characteristics that make it a good candidate for the study of natural pathosystems. Spores are easily collected and stored, and artificial inoculation of seedlings with good control of dosage is possible. Galls are easily counted and provide a measure of the amount of disease, as well as of the damage done by the disease. The major disadvantage is the long time period required for most types of experiments.

One of the central questions about natural pathosystems is the extent to which major resistance and virulence genes in the host and pathogen, respectively, play a role in the epidemiology of

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the disease. On the one hand, natural pathosystems may be essentially horizontal, such that the relative resistance of individual host plants does not depend on the pathogen race used. In such a horizontal system, resistance and aggressiveness are often thought to be conditioned by many minor (i.e., essentially undetectable) genes. On the other hand, natural pathosystems may also be "natural multilines", the host population consisting of a (possibly large) number of distinct varieties, each susceptible to a (possibly small) subset of the pathogen races present in the pathogen population. These are two extreme positions. A mixture of both horizontal and vertical effects probably occur in most natural systems. In addition, major resistance genes interacting with pathogen virulence genes need not act in the all-or-nothing fashion of the traditional gene-for-gene system. They may instead affect the infection rate in a significant way. Conifer pathosystems such as the one described here differ in a major way from most agricultural systems. The host is much more variable. There is not a single pair of genetically identical lodgepole pine trees in the world. At the very least, this means that major genes act in very different genetic contexts.

There is some evidence for interaction between distant provenances of pine and the rust (van der Kamp 1988; Kojwang 1989). Such interactions may be caused by major genes for resistance and virulence present in some local pathosystems and not in others. Movement of major genes for resistance, and their complementary genes for virulence, from one area to another may introduce a temporary interaction in a local pathosystem until such genes become universal in such pathosystems.

The question to be addressed in this paper is whether major resistance and virulence genes are common enough to play a significant role in the epidemiology of the disease in a local pathosystem. A further question, namely, whether there are rare major resistance and virulence genes that may have an inconsequential effect in the natural system, but that may be promoted by selection for resistance, can never be answered with certainty in the negative. Trials such as the one described here may or may not detect such rare genes.

In this paper we describe a study of the interaction between pine clones and single-gall inocula. The latter are assumed to be genetically uniform.

METHODS

Preparation of Clones

Scions were collected from two adjacent stands near Prince George, B.C., in April 1983. The first of these was a block in an experimental spacing trial planted in 1967 as 2-0 stock raised from local seed; the other was a natural stand regenerated after a fire in 1961. Both are described in some detail in van der Kamp (1988). The scions were grafted onto 2-year-old rootstock derived from open-pollinated seed collected in the same stands. In 1987, at the time of inoculation, these clones had a physiological age of 22 and about 25 years for the two origins respectively.

Collection of Inoculum

The inoculum used in the experiment came from the same two stands described above. Four large galls showing abundant spore production and no evidence of hyperparasites were selected. Spores were extracted in the field, sieved, and stored at −3°C for 11 days until use. Spore source 1 came from the parent of clone 15 in the natural stand, and source 3 from clone 2 in the plantation. The other two were collected from two separate trees not represented in the clone collection.

Inoculation Procedures

Potted ramets were maintained in cold storage (2°C) in the dark from March 15 until May 10, 1987, to delay shoot elongation until spores could be collected at Prince George; they were then brought outside to allow normal flushing. By June 6 the new shoots had reached about 75% of their final length, and needles were just beginning to emerge from their fascicle sheaths. The potted clones were then brought inside, and the shoots to be inoculated enclosed in plastic bags. Bags were then removed one at a time, and the appropriate spore source dusted onto the unwounded new shoot using a small brush. The shoot was then misted with distilled water and the bag replaced. Controls consisted of bagged and misted shoots without spores. All remained uninfected after 13 months. After 2 days at about 18°C the bags were removed and the trees brought outside. Early symptoms were recorded 4 and 8 weeks after inoculation. The final assessment of the number of galls took place in July 1988, 13 months after inoculation. Spore-by-clone combinations were mostly represented by four or five replicates. In clones 10, 14, 15, and 16, however, the number of replicates ranged between 6 and 20.

RESULTS

Typical early symptoms consisting of small red flecks were observed 8 weeks after inoculation on many shoots. The frequency of these symptoms, however, was much lower than that usually observed on 1- and 2-year-old seedlings inoculated in the same way. Development of adventitious juvenile shoots from needle fascicle meristems located on infected tissue, a common occurrence on young seedlings, was not observed. Gall formation was also slower than on younger seedlings; no galls were visible in October 1987. On seedlings many galls would have been visible as distinct swellings at that time.

The average number of galls per inoculated shoot for each clone-by-spore source combination is shown in Table 1. Table 2 presents an ANOVA of these data, and shows a significant interaction between the effects of clone and spore source. Perusal of Table 1 shows that this interaction is largely attributable to four clones (3, 9, 11, and 12) that deviated from the normal pattern of disease severity, and to the two clones that remained uninfected (5 and 7, both derived from trees that were uninfected in the field; two other uninfected trees yielded susceptible clones). For the remaining 10 clones, the degree of infection followed the spore source average rather well. Of the 64 spore source by clone combinations, 17 remained uninfected. Eight of these represented the two uninfected clones.

DISCUSSION

The variation between clones (0 to 2.89 infections per shoot, half the clones having <1.0 galls per shoot) was not unlike the variation found between individual trees in the field. The difference in the overall performance of the spore sources represents a problem. The average rate of infection by source 3 was considerably less than that of the other three sources. If, as seems likely, spore lines reproduce asexually, a spore line with such a low relative rate of infection would soon disappear from the population. Several explanations are possible however. First of all this experiment measured the infection resulting from a uniform amount of inoculum. The volume of spores produced by individual galls varies considerably. It may be that the low infection rate by spore source 3 is counterbalanced by high spore production. There may also be a spore source and environment interaction, such that source 3 might be much more pathogenic (relative to the other sources) in environments other than the one used in this study.

Table 1. Average number of galls per shoot for 16 clones of lodgepole pine inoculated by four single-gall western gall rust spore sources

Clone number	Spore source 1	2	3	4	Clone means
1	2.25	4.75	1.00	2.25	2.56
2	0.20	0.50	1.25	1.40	0.84
3	0.00	0.50	0.40	1.50	0.60
4	1.50	0.00	0.33	0.00	0.46
5	0.00	0.00	0.00	0.00	0.00
6	2.33	5.00	3.00	1.25	2.89
7	0.00	0.00	0.00	0.00	0.00
8	1.00	0.50	0.00	0.00	0.37
9	2.00	3.50	0.50	0.00	1.50
10	2.45	2.94	0.89	3.24	2.38
11	3.00	0.00	0.00	0.00	0.75
12	0.00	3.50	0.25	2.33	1.52
13	1.80	1.80	1.60	4.80	2.50
14	0.60	1.14	1.00	1.00	0.93
15	1.00	1.17	0.60	1.57	1.08
16	2.00	2.56	0.38	0.57	1.38
Mean	1.26	1.74	0.70	1.29	1.24

Table 2. Analysis of variance of number of galls per shoot on 16 lodgepole pine clones inoculated with four single-gall western gall rust spore sources

Source	d.f.	F	Test term (months)	% of total variance
1. Inoculum	3	4.86**	3	5.6
2. Clone	15	3.70**	3	20.9
3. I × C	45	1.66**	4	16.9
4. Error[a]	250			56.6

[a] Error mean square = 2.5086.

Finally, the spore sources used in this experiment were derived from single galls. Variation in spore quality resulting from degree of maturation at the time of collection, host effects, or contamination, may possibly account for some of the differences in the average behavior of the various sources. True replication of spore sources would involve raising inoculum on seedlings inoculated with single-gall spore sources.

A classical multiline is thought to reduce infection because of a spore-diluting effect. If different host individuals are intimately mixed, and only a small proportion of the total host population is susceptible to any one of the rust races present in the pathogen population, then many or most of the spores produced on a susceptible host will land on resistant individuals and thus not contribute to the ongoing epidemic. In this pathosystem such a spore-diluting effect was not very obvious. A few host and clone combinations showed a classic interaction (e.g., clone 11 and 12 with all four spore sources, and clone 3 and 4 with source 1 and 2), but most did not. Individual spore sources caused infection on 62.5 to 75% of the clones tested (71 to 86% if the uninfected clones are ignored). The lodgepole pine-western gall rust pathosystem does not appear to be a natural multiline in the classical sense. The four spore sources represent a small sample of the pathogen population. A larger sample might have detected classical interactions involving most or even all of the clones; however, the overall pattern, namely that most clones are susceptible to most races, is well established by these results.

The analysis of variance presented in Table 2 shows a considerable interaction effect. Several of the clones tested in this experiment were distinctly more susceptible to some, and more resistant to other, rust isolates than the general host population. If one generalizes from these limited experimental results, the following picture emerges. Individual trees vary considerably in their susceptibility to a natural multirace rust inoculum. Thus when a new plantation or a natural stand newly established after a fire, is exposed, under conditions favorable to the disease, to the mixed inoculum produced in surrounding pine stands, some trees are quickly and heavily infected, others are lightly infected, while some will be so resistant that they remain free of infection for a long time. Once a tree has become infected, the spores produced on that tree form a disproportionately large part of the inoculum to which the tree is exposed from then on. The extent will depend on the contribution to new infection of the general spore cloud relative to the infections already established on a tree. Thus, if among the rust races on that tree there are some to which that tree is particularly susceptible, such races will become more common on the tree in question. Table 1 gives an indication of the degree of specialization. The overall infection rate was 1.24 galls per shoot. The average infection level for the 16 cells that represent the most successful race on each of the clones is 2.40 galls per shoot. The 16 least successful races averaged 0.41 galls per shoot. A larger selection of spore sources might well increase these differences.

Elsewhere in these proceedings (van der Kamp 1990) it is argued that the random placement of spores on susceptible tissues results in a source of variation in number of infections per tree (or, in this context, shoot) that is irreducible by careful control of environmental factors. The number of infections that appear on a unit of tissue will follow a Poisson distribution, with the mean (and variance) equal to the average infection rate for that tissue. In this experiment, the average variance over mean ratio of the 47 spore source by clone combinations that were infected, was 0.985, and not significantly different from unity. This suggests that the variation encountered was largely attributable to the random placement of spores on the inoculated shoots, and therefore not reducible by more careful control of experimental procedures and conditions. It also means that the failure of infection in some spore source by clone combinations may be a chance effect that is to be expected. For instance, if the infection rate of a particular combination is 0.5 galls per shoot, then with four replicates, the probability of all being uninfected is $(e^{-0.5})^4$ or 0.135.

In conclusion, the lodgepole pine-western gall rust pathosystem appears to be a mixed system in which both horizontal and vertical effects occur, but in which the former predominate. Selection from such a system of a small number of clones may well result in a corresponding selection for a few rust races that are particularly well adapted to these clones. On the other hand, selection of a large number of individuals (for resistance or other purposes) for use in seed orchards is not likely to disturb the equilibrium between the host and pathogen to any great degree.

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SUSCEPTIBILITY OF PONDEROSA PINE TO WESTERN GALL RUST

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ABSTRACT

Differences in susceptibility among provenances of ponderosa pine were observed in response to artificial inoculation of western gall rust. The northern inland populations were highly resistant to gall rust. Populations nearest the source of the inoculum were most susceptible. Results also showed substantial genetic variation among and within families. Family h^2 was 0.84.

INTRODUCTION

Western gall rust caused by *Endocronartium harknessii* is a relatively minor disease of northern populations of ponderosa pine (*Pinus ponderosa* var. *ponderosa*). Most damage is to young trees and is frequently confined to branch galls (Leaphart 1955; Peterson 1959).

Thomas et al. (1984) reported that ponderosa pine from eastern Washington and Oregon, Idaho, and central and western Montana were most resistant, and populations from the Colorado plains and the southern Rocky Mountains were most susceptible to gall rust. A more detailed study of central Idaho populations that had been artificially inoculated with a high number of spores revealed many highly susceptible populations (Hoff 1986). Infection levels among these populations ranged from 65 to 95%.

The purpose of the study reported here was to assess variation patterns in susceptibility to western gall rust in populations of ponderosa pine from northeastern Washington, northern Idaho, and western Montana, and to assess the level of genetic variation within and among families of four widely spaced populations.

MATERIALS AND METHODS

Seeds from 125 populations from northeastern Washington, northern Idaho, and Montana west of the continental divide (Fig. 1) and covering the elevation range of the species were included in this test. The seeds were sown in 20.3 cm^2 (8 sq. in.) containers and grown and overwintered in a shade house at the Intermountain Research Station's Forestry Sciences Laboratory at Moscow, Idaho (lat. 46°44′N, long. 117°0′W, elevation 808 m (2650 ft)).

The experimental design was: 125 populations, 10 seedlings per population per replication, three replications. Seedlings were grown in row plots. Total seedlings were 1250 per replication and 3750 for the test.

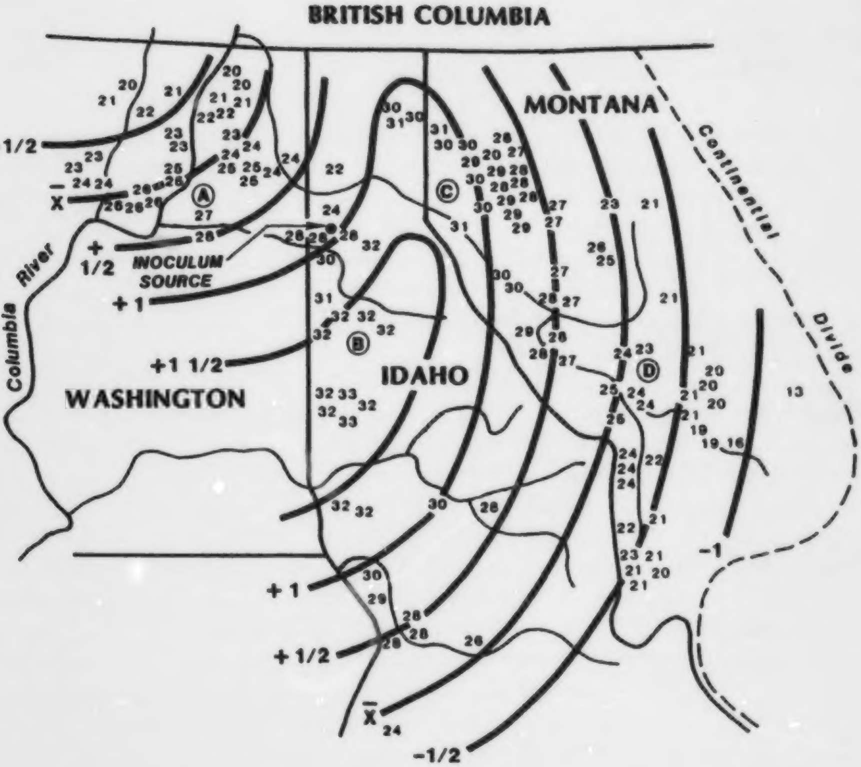

Figure 1. Locations of populations of ponderosa pine; numbers represent predicted values at the mean elevation, letters represent geographic regions used in the analysis, and lines represent levels of equal predicted infection levels. The intervals between lines equal 1/2(lsd(0.2)) and are positive or negative deviations from the mean value of all populations.

In May, the seedlings were moved into a mist chamber in preparation for inoculation. The seedlings were just beginning their second growing season and needles were just breaking out of the fascicle sheaths.

Fresh inoculum was collected from at least 50 galls from a young ponderosa pine stand located at the Lone Mountain Tree Improvement Site (lat. 47°54'N, long. 116°49'W, elevation 759 m (2488 ft)) about 16 km (25 miles) north of Coeur d'Alene, Idaho. Inoculation was completed in a mist chamber on May 30, 1984, by blowing dry spores over the seedlings using an air-sprayer adapted from an Erlenmeyer flask. The level of inoculation was 20 000 spores per seedling. After several weeks of acclimation seedlings were moved to a nursery at Priest River Experimental Forest (PREF) (lat. 48°21'N, long. 116°52'W, elevation 732 m (2400 ft)), where they were planted at a 15 × 15 cm (6 × 6 in.) spacing in beds 76 cm (30 in.) wide. The 10 seedlings for each provenance in each replication were planted in two adjacent rows of five trees each. Planting dates were June 19 and 20, 1984.

Seed for the family test was collected from four widely spaced stands. Twenty trees were selected randomly from each stand. The sowing of the seed, growing, inoculating, and planting of the seedlings into the PREF were the same as for the provenance test.

Inspections for rust infections were made for each seedling at 3 months, 15 months, 27 months, and 39 months after inoculation. Each seedling was tallied for the presence of galls.

Analysis of Provenance Test

Analysis of variance was used to determine significant differences among stands for the degree of infection (presence of galls).

Simple and multiple regression models were used to relate susceptibility to elevation and geographic location of the seed source. The independent variables were elevation, latitude, longitude, northwest departure, southwest departure, azimuth from the inoculum source, and distance from the inoculum source. Northwest (latitude × longitude) and southwest (1/latitude × longitude) departures were derived by rotating grid of latitude and longitude by 45°. Squares of the independent variables were added to accommodate the possibility of nonlinear patterns of variation. Therefore, 14 independent variables were included in a stepwise regression for maximizing R^2 (SAS 1982). Further, the geographic variables were nested within four geographic regions: northeastern Washington, most of Idaho, northwestern Montana including the most northerly stands in Idaho, and middle to southwestern Montana (Fig. 1).

Analysis of Family Data

Analysis of variance was used to determine significant differences among families for the degree of infection. Genetic variation was expressed in terms of heritabilities and genetic gain. Genetic variances were adjusted by the assumed level of inbreeding (10%).

RESULTS

The analysis detected highly significant differences among populations for the degree of infection (Table 1). The average level of infection 39 months after inoculation was 28%; populations varied from 3 to 63%.

There were no strong associations between susceptibility and the environmental factors (Table 2). The R^2 for level of susceptibility and elevation was 0.15, the highest single association. The R^2 for the best fit stepwise multiple-regression model using all environmental factors with the level of infection was 0.26. Figure 1 shows geographic lines at a constant elevation (average elevation for all stands). The distance between contour lines equals 1/2(lsd(0.2)). When comparing populations from the same elevation, populations from the west central area--the area near the origin of the rust inoculum--displayed the highest level of infection. From here, the level of infection decreased in all directions.

Figure 2 shows the association between elevation of the seed source and susceptibility. The elevation clines are keyed to the four geographic areas shown in Figure 1. They all indicate the degree of infection increases as elevation of seed source increases, although the relationship tails off at high elevations.

Table 1. Analysis of variance of the level of infection by western gall rust of populations of ponderosa pine

Source of variance	d.f.	Mean square	F
Replication	2	0.003	
Populations	124	0.090[a]	2.1
Replications × populations	248	0.042	

[a] Significantly different at 1%.

Table 2. R^2s between rust factors and various environmental factors

	Environmental factors[a]						
Symptom of infection	El.	Lat.	Long.	NW	SW	Az.	Dist.
Level of infection	0.15[b]	0.08b[b]	0.05[b]	0.09[b]	0.02	0.05[b]	0.20

[a] El. = elevation, Lat. = latitude, Long. = longitude, NW = northwest departure, SW = southwest departure, Az. = azimuth, Dist. = distance from the inoculum source in miles.

[b] Significantly different at 1%.

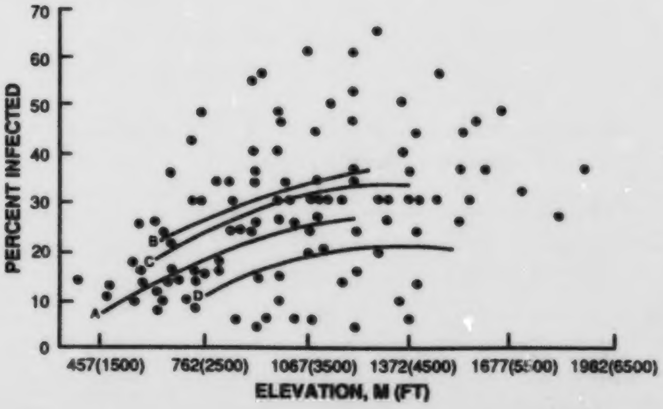

Figure 2. Actual percentage infection plotted against elevations. Lines A to D represent elevation clines for the geographic areas indicated in Figure 1.

The differences among and within families were highly significant (Table 3). Family heritability was 0.75. At one unit of i (selection intensity), 12% gain could be made for selecting of best families.

DISCUSSION

The general level of susceptibility of this collection of ponderosa pine populations to western gall rust was low; however, several populations were highly susceptible.

Much of the variation among populations appeared to be random. Nonetheless, susceptibility among populations could be partly explained by geography. The R^2 for elevation was 0.15. Generally as elevation of populations increased so did the level of infection; however, the relationship was curvilinear with the level of infection leveling off at about 1220 m (4000 feet). That low elevation sources are less susceptible is not surprising, since weather conditions appear to be ideal for infection with the probable result of higher selection for resistance.

The multiregression that gave the best fit used a combination of elevation, azimuth, distance from inoculum source, and southwest departure. These accounted for about 50% of the variation in infection. Most surprising was that the level of infection was higher near the inoculum source, but then decreased in all directions from there. Powers and Matthews (1980) reported the same relationship with loblolly pine-fusiform rust. Possibly resistance to gall rust is already so high in ponderosa pine that there is no selection pressure for increased resistance. Thus the fungus and ponderosa pine in this part of its range have reached a balance that has resulted in the fungus having a slightly higher virulence to the local populations. Powers and Matthews (1980) thought that the fusiform rust fungus has adapted to loblolly pine and consequently produced races that are more virulent in the local sites. I find this explanation easier to accept with a system like loblolly pine-fusiform rust, which has been excessively disturbed, than with a system like ponderosa pine-western gall rust, which has had comparatively little disturbance.

The level of genetic variation within stands of ponderosa pine and within the randomly chosen individual trees indicates that substantial gains could be made in selecting for resistance.

Table 3. Analysis of variance of the level of infection for families of ponderosa pine by western gall rust

Source of variance	d.f.	Mean square	F
Replication	4	1.58	
Population	3	10.22[a]	44.4
Family in population	76	1.53[a]	6.7
Experimental error	316	0.23	
Within plot	3547	0.20	

[a] Significantly different at 1%.

In terms of tree improvement, there appears to be little danger in moving seed from one locality to another unless a particularly susceptible stand is chosen as the source of seed. Thus, it is important for forest managers to closely inspect stands before seed collection to determine the level of susceptibility to western gall rust. Further, it is obvious that some individuals are highly susceptible, therefore care must be taken in selecting seed trees for natural regeneration.

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A JOINT U.S.–CANADA BLISTER RUST "RACES" TEST ON *PINUS MONTICOLA*: FIRST-YEAR RESULTS

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INTRODUCTION

White pine blister rust (*Cronartium ribicola* J.C. Fisch. in Rabenh.) arrived in western North America at Vancouver, British Columbia (B.C.), about 1910 on infected seedlings from Europe (Boyce 1948). The rust's method of arrival and duration at the launching point would affect the gene complement reaching Vancouver, but probably it was a limited subpopulation of that available in Europe.

Rust-spread from Vancouver was rapid; by 1928 it occurred throughout much of the main commercial range of eastern white pine (*Pinus monticola* D. Don) (Mielke 1943). The epiphytotic presence in the U.S. Inland Empire was "the world's most spectacular" (Bingham 1983). Such rapid spread from a single source could produce differences in the rust due to founder effects in the open landscape offered by the highly susceptible hosts.

Evidence of racial variation in the rust is reported by several workers, including differences in infectability of alternate hosts (Stephan and Hyun 1983) or severity of reaction on the alternate hosts (Anderson and French 1955) and susceptibility of pines (Delatour and Birot 1982; Yokota 1983; Stephan 1986), rust virulence "hot spots" (Kinloch 1981; McDonald et al. 1984), and intrapopulation variability in the rust expressed as spot color on pines (McDonald and Hoff 1975).

Racial variation in blister rust is suggested by disease levels in three plantations on Vancouver Island, B.C. At age 11, one plantation of 80 trees from rust-resistant Idaho parents was 42% stem-cankered or dead, another of 65 trees from the same seed source was 75% infected in the stem or dead, while 52% of a nearby plantation of unscreened local stock was similarly infected (Hunt and Meagher 1985). This same Idaho seed source produced 35% or less infected saplings at a comparable age under heavy rust pressure in northern Idaho (R. Hoff, USDA Forest Service, Moscow, Idaho, personal communication 1984).

Since white pine is gaining interest among foresters in B.C. due to both its high value and its resistance to certain root rots, importation of rust-resistant seed from Idaho is an attractive option if 1) genetic variation in the species for non-rust adaptive features is low, as concluded by Steinhoff et al. (1983) and Rehfeldt et al. (1984), and 2) variability in the rust is small. The former problem is being studied for B.C.'s white pine range at the University of British Columbia. The latter problem is being studied to determine the differences, if any, in infection success and virulence on *P. monticola* families by blister-rust populations in British Columbia and northern Idaho.

MATERIALS AND METHODS

Seedlots

Twenty-four seedlots from four regions were assembled to represent white pine populations in the portion of its range of greatest potential interest to B.C. Six seedlots selected in three pairs from coastal B.C., six from coastal U.S. (Washington and Oregon), six in the three pairs from interior B.C., and six from interior U.S. (northern Idaho, 3 from one location) were used (Fig. 1). Where possible, seedlots possessing known resistance reactions and levels were selected (Table 1).

Seedlings

Following a warm-cold stratification (Edwards et al. 1987), seeds were sown singly in 65.6-cm^3 plastic containers (Ray Leach fir cells) containing a medium composed of peat and vermiculite (3:1 by volume) supplemented by 3000 g of coarse dolomite limestone, 130 g of fritted trace elements (FTE 503), and 6500 g of 9-month-release Osmocote 18-6-19 fertilizer per cubic metre.

Seeds were sown in family (seedlot) lots of 200 containers (one rack), then were covered with fine granite grit. Where possible, sufficient seeds were sown to provide 400 seedlings per seedlot. Racks were misted frequently during the first 30 days when most germination occurred, then watered as needed. Fertilizers (20-20-20 NPK at 0.5 g/L) were applied twice weekly through the irrigation system; weekly flushing reduced salt buildup. Racks were shaded lightly in a cooled greenhouse during the germination and early-growth phases. Shading and cooling were reduced by mid-summer, and fertilizer formulation was changed to 0.5 g/L of 10-15-16 once weekly in late summer to induce bud-set and hardening-off. The seedlings were moved to a fiberglass-covered, open-sided shadehouse in September where they remained until prepared for rust inoculation in 1987. Fertilizers were added to the irrigation water in the second year, using the same formulation and schedule as in the previous year.

Prior to rust inoculation, seedlot replicates were established in a randomized pattern for inoculation by a rust source. Up to 10 replicates, consisting of up to 10 seedlings each per seedlot, were established for each rust source. One replicate occupied 2.5 racks; thus, successive replicates might share one rack. The order of seedlots was randomized separately for each replicate in rust. All racks were labeled securely with rust, replicate, and seedlot numbers.

Rust Sources and Culture

Four rust sources were used (Fig. 1). Rusts were obtained in the spring of 1987 by collecting and mixing similar volumes of aeciospores from several infected pines per source. The coastal-mix (Whistler, in Fig. 1) rust was created by pooling aeciospores from several sites on central-northern Vancouver Island and the B.C. mainland. *Ribes* plants in isolated natural groves or in cultivated *Ribes* gardens that had remained free of infection for a minimum of 3 years were inoculated with the aeciospores. The resulting infections were monitored throughout the summer to follow their development and intensify the infection, where necessary, by wafting urediniospores from infected plants to others nearby.

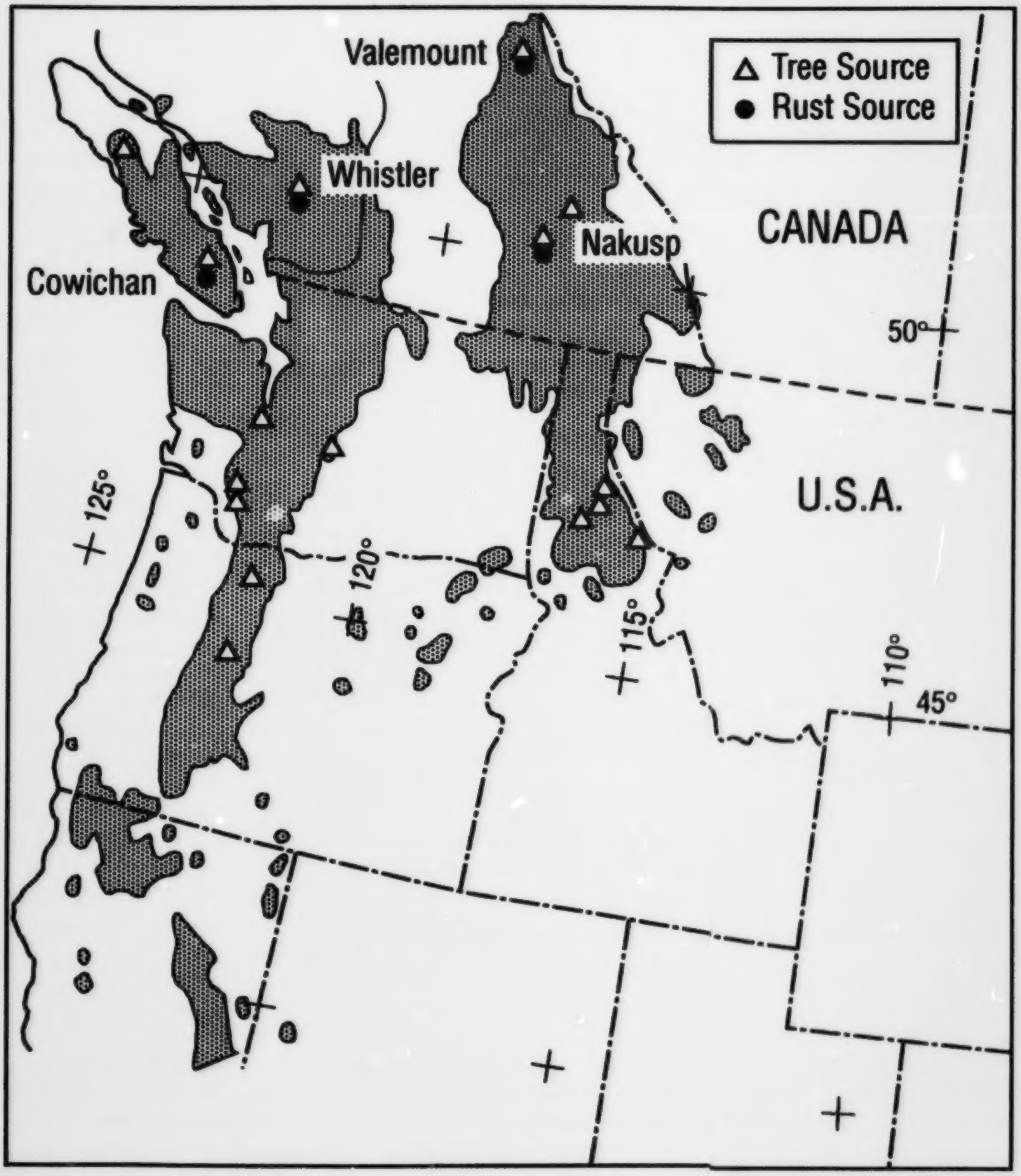
Valemount
Δ Tree Source
● Rust Source
Whistler
Nakusp
CANADA
Cowichan
50°
U.S.A.
125°
120°
115°
110°
45°

Figure 1. The northern range of *Pinus monticola* showing the sources of seed (tree source) and rust (rust source).

Table 1. Origin and rust-resistance mechanism(s) of *Pinus monticola* seedlots used

Country	Zone	Agency[a]	Parent tree no.	Seedlot no.	Rust defense(s)[b]
Canada	Coast	ForCan	Whistler B	2249	None
			Whistler D	2251	None
			46 -19 Creek	2336	None
			57 - C.L.R.S.	2343	None
			230 Woss	2414	None
			228 Woss	2426	None
	Interior	ForCan	Valemount 5	2293	None
			Valemount 4	2296	None
			Mt. Revelstoke E	2279	None
			Mt. Revelstoke B	2349	None
		Westar	9042 Arrow Lks.	2494	?
			9043 Arrow Lks.	2495	?
United States	Coast	U.S.F.S. (a)	05014-102	2443	HNF, NS &/or SS
			18034-725	2444	RNF, NS or SS
			06025-526	2445	RNF, NS or SS
			03124-357	2446	RNF
			17114-825	2447	RNF
			03014-124	2448	HNF, NS &/or SS
	Interior	U.S.F.S. (b)	4056	2451	?
			3208	2452	?
			3300	2453	?
			3310	2454	?
			5168	2455	?
			290	2456	?

[a] ForCan = (Forestry Canada, Victoria, B.C.); Westar = (Westar Corp., Nakusp, B.C.); U.S.F.S. (a) = U.S. Forest Service, Cottage Grove, Oregon; U.S.F.S. (b) = U.S. Forest Service, Moscow, Idaho.

[b] Based upon tests by the cooperating agency. None = no data, ? = data incomplete, HNF = high needle (spot) frequency, RNF = reduced needle spot frequency, NS = early shedding of spotted leaves, SS = rust arrested in leaf bundle short shoot.

Seedling Inoculation

Once basidiospores were produced on the *Ribes* plants, sufficient leaves were collected from each rust source to cover screens suspended over the racks of seedlings to be exposed to that specific rust "race". When more than one rust source was in the inoculation chamber at the same time, compartments allocated to different sources were shrouded to reduce possible cross infection. Hunt (1988) gives details of the procedure to obtain adequate infection. Following inoculation, all stock was returned to the Pacific Forestry Centre, Victoria (48°30′N, 123°25′W), for the winter.

Rust Spore-Cast Monitoring

Spore density at seedling canopy level was monitored on 2 or 3 glass slides for each rust source. Spore counts were made in ten fields per slide, accumulated by slide, and expressed as spores per cm^2. Seedling racks were removed from the chamber when the 3000 spores per cm^2 target mean was approached, or shortly after it was exceeded.

Infection Assessment

Prior to examination for rust symptoms, all seedlings were assembled by replicate within seedlot by rust source to simplify records. In May of 1988, 8 months after being exposed to rust, each living seedling was examined for blister-rust spots on the needles. The categories of spot size used were

1) Fleck: a spot centered in the stomatal bands but not extending the width of the leaf,
2) Small spot: a spot extending the width of the leaf but not longer than leaf width, and
3) Big spot: a spot extending the width of the leaf and longer than leaf width.

Colors were assigned also; flecks were described as only yellow, orange or red, whereas small or big spots were described as

Yellow: lemon to butter-colored, margin regular;
Green-yellow: yellow and green tissue intermingled, margin irregular;
Orange: no red showing, margin regular;
Yellow-brown: brown usually in the central area, margin regular;
Brown: spot wholly brown;
Green-brown: brown and green intermingled, margin irregular;
Green-red: red and green intermingled, margin irregular;
Red-brown: red and brown intermingled, margin irregular;
Red: pink to ruby red spot, margin regular.

Thus, a seedling could show up to three fleck colors and up to nine colors in each of the small- and big-spot categories.

Seedling Transplanting and Maintenance

After inspection, all stock was planted in a holding area near Victoria for observation of rust development. Stock was transplanted in numerical sequence by replicate in seedlot in rust source in the transplant beds. The beds were irrigated by a trickle system, fertilized annually using general-purpose N-P-K formulations, and treated against weeds to maintain healthy stock.

Subsequent Inspections

Seedlings are being evaluated annually to monitor rust development and tree vigor.

Data Compilation and Analysis

Data were summarized by replicate within seedlot by rust to determine

1) percentage spotted, including fleck;
2) percentage spotted by spot size, including flecks; and
3) spots per seedling by size class, including flecks, and total.

Analyses of variance were conducted on spot counts per tree and percentage of trees spotted, treating all effects, but replicates, as fixed. Selected means were tested by Student's *t* (Zar 1984).

RESULTS

Seedlot Representation

Due to differences among seedlots in germination and survival, both the number of seedlings per replicate (2-10) and, to a lesser degree, the number of replicates per seedlot (3-9) varied within a rust source.

Spore-cast Monitoring

The time required to reach the target of 3000 basidiospores/cm^2, or until spore release stopped, was 10-120 h. Average spore densities attained were 5330, 3860, 2970, and 2800/cm^2 for the Cowichan, Valemount, coastal mix, and Nakusp sources, respectively. Larger differences occurred among sampling points within source; e.g., an eightfold difference (370-3110/cm^2) occurred between samples in the Nakusp source. Other values were within 7-51% of the mean by source. The highest spore density (7840/cm^2) was found on a slide from the Cowichan source.

Inoculum Density: Spotting Success

Mean infection percentage per rack (by averaging spotted percentage among seedlots) was examined to determine the effect of the variation in spore density within the chambers. The lowest (370/cm^2) and highest (7840/cm^2) spore densities produced little difference in percentage spotted per rack (71.6 ± 31.0% versus 85.2 ± 16.0%). Since those came from different rust sources, comparison was made among racks exposed to the same source but receiving different spore densities. Mean spotted level from 2510 spores/cm^2 was 77.6 ± 23%, and from 7840 spores/cm^2 it was 85.2 ± 16.0%; the values are not statistically different ($p \geq 0.05$).

Inoculum Density: Spots per Seedling

Mean spots per seedling were determined by family for the racks reported in the preceding section. The lowest spore density (370/cm^2) produced the lowest spotting value (2.64 ± 2.36 per seedling), while the highest spore density (7840/cm^2) produced a higher spotting value (6.34 ± 8.80 per seedling). However, the lowest spore density (2510/cm^2) in the rust delivering 7840 spores/cm^2 resulted in 7.10 ± 5.12 spots per seedling.

Spot Sizes

All spot-size classes were produced by all rust sources in most seedlots. Flecks were not found in five replicates involving only three seedlots. Three of these cases occurred in the two seedlots from northern Vancouver Island (Woss) exposed to the Cowichan rust. The other two replicates involved the Mount Hood, Oregon, seedlot and a Woss seedlot exposed to the Nakusp rust. Therefore, in 91 of 96 replicates (94.8%) all spot sizes were produced by all rusts. Analysis of spot size (fleck, small or big spots) showed that rust source, parent-tree origin, and their interaction were highly significant ($P < .01$) for all spot sizes. The effect of parent tree in region (e.g., B.C. interior, U.S. coastal) was significant for only big spots. The coastal-mix rust source was chiefly responsible for the source significance, whereas the interior seedlots, and their response to the coastal rusts, were mainly responsible for the region and the region × source interaction. Figure 2 presents mean spot frequency, excluding flecks, by size in color for interior B.C. seedlings exposed to each rust source. The preponderance of green-yellow spots following inoculation by coastal rusts (coastal mix and Cowichan) is apparent.

Spot Colors

Less balance occurred in spot color than in spot size. Most flecks were yellow, whereas most spots were green-yellow. Green-red spots were least common. Seed source affected color distribution; interior seedlings displayed mainly yellow to green-yellow spots, whereas coastal seedlings showed more balance among the color classes (Fig. 3). Color patterns within rust source and parent region were the same for both small and big spots.

Percentage of Seedlings Spotted

Infection level by rust source was satisfactory, averaging 88.5% for the coastal mix source, 75.4% for the Cowichan source, 74.5% for the southern interior (Nakusp) source, and 78.4% for the northern interior (Valemount) source.

More variability occurred among seedlots; infection ranged from 30.7% in seedlot 2454 from the Saint Joe National Forest, Idaho, by the Nakusp rust to 98.6% in seedlot 2446 from the Gifford Pinchot National Forest, Washington, by the coastal-mix rust.

Infection success among replicates was very high; only two replicates containing 10 and 3 seedlings, respectively, and involving different seedlots were not spotted 8 months after inoculation. All other seedlots in the same two racks were spotted, averaging 91% and 74% spotted per family by rack. Twenty months after inoculation the unspotted replicates were free of rust.

All Spots per Seedling

Total spots and flecks per seedling ranged from 0 to 105. Figure 4 presents all spots per seedling by rust source in seedlot, grouped by region of origin. The mean number of spots plus flecks per seedling by region was 6.91, 5.65, 4.64, and 4.16 for the B.C. interior, B.C. coast, U.S. interior, and U.S. coast, respectively. Only the last two values did not differ significantly. The coastal-mix rust produced more spots per tree (7.77) than the others (Nakusp 4.89, Valemount 4.47, Cowichan 4.45), which did not differ. The highest mean value of spots per tree (20.78) was produced by the Nakusp rust (no.

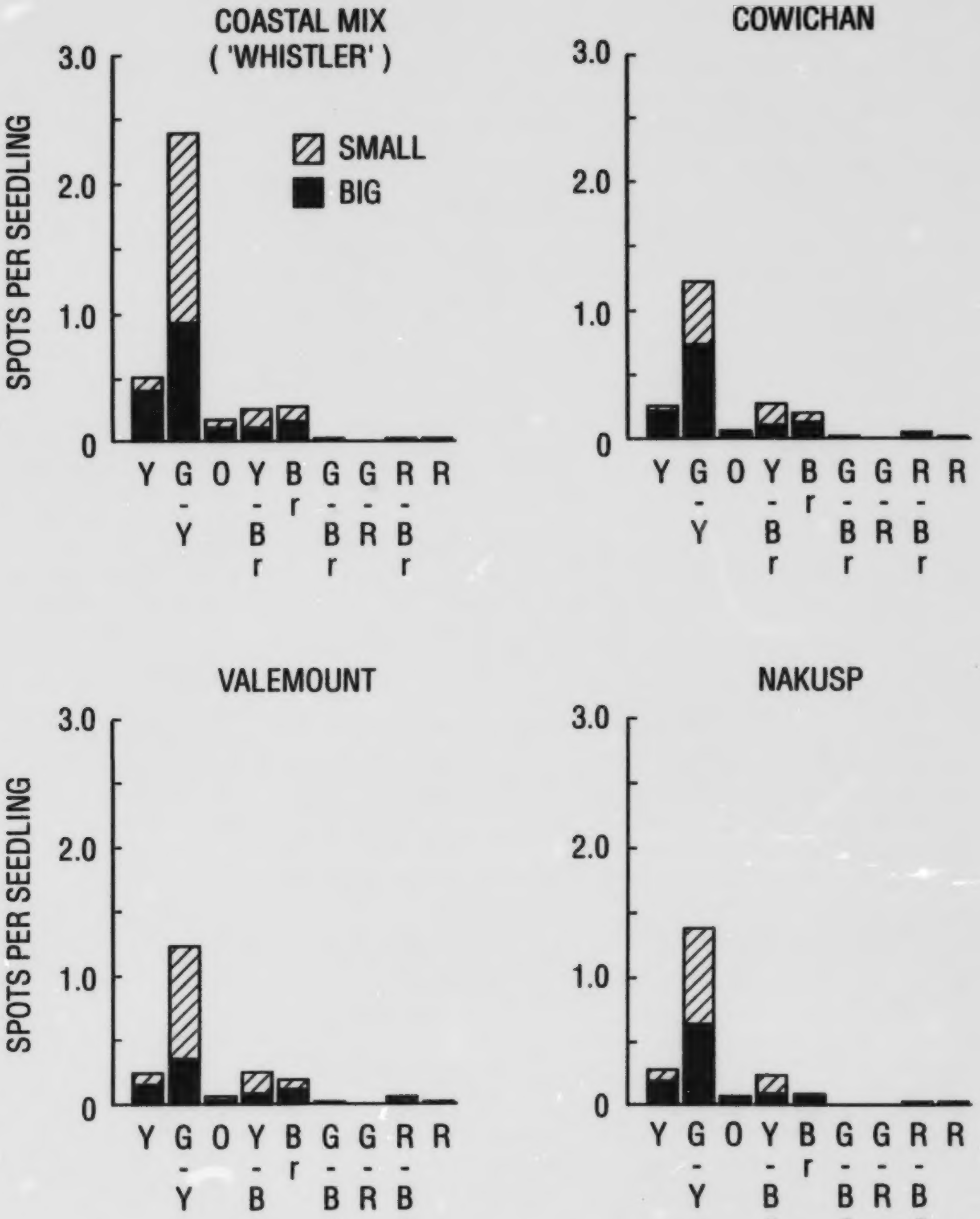

Figure 2. **Effect of rust source (race) on spot size and color balance in seedlots from the British Columbia interior.** Spot colours: Y (yellow), G/Y (green-yellow), O (orange), Y/Br (yellow-brown), Br (brown), G/Br (green-brown), G/R (green-red), R/Br (red-brown), R (red).

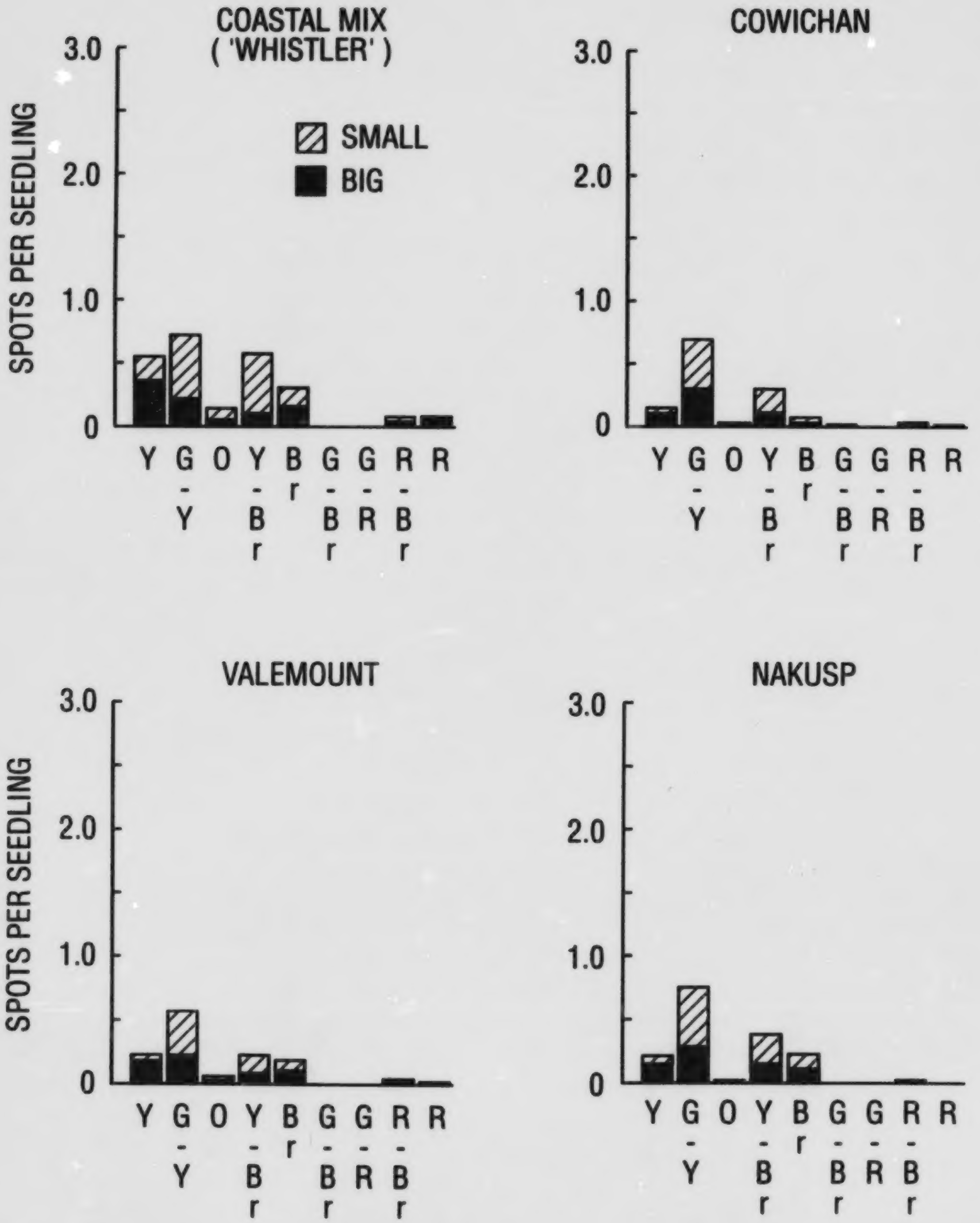

Figure 3. Effect of rust source on spot size and color balance in seedlots from the United States coast. Spot colors as in Figure 2.

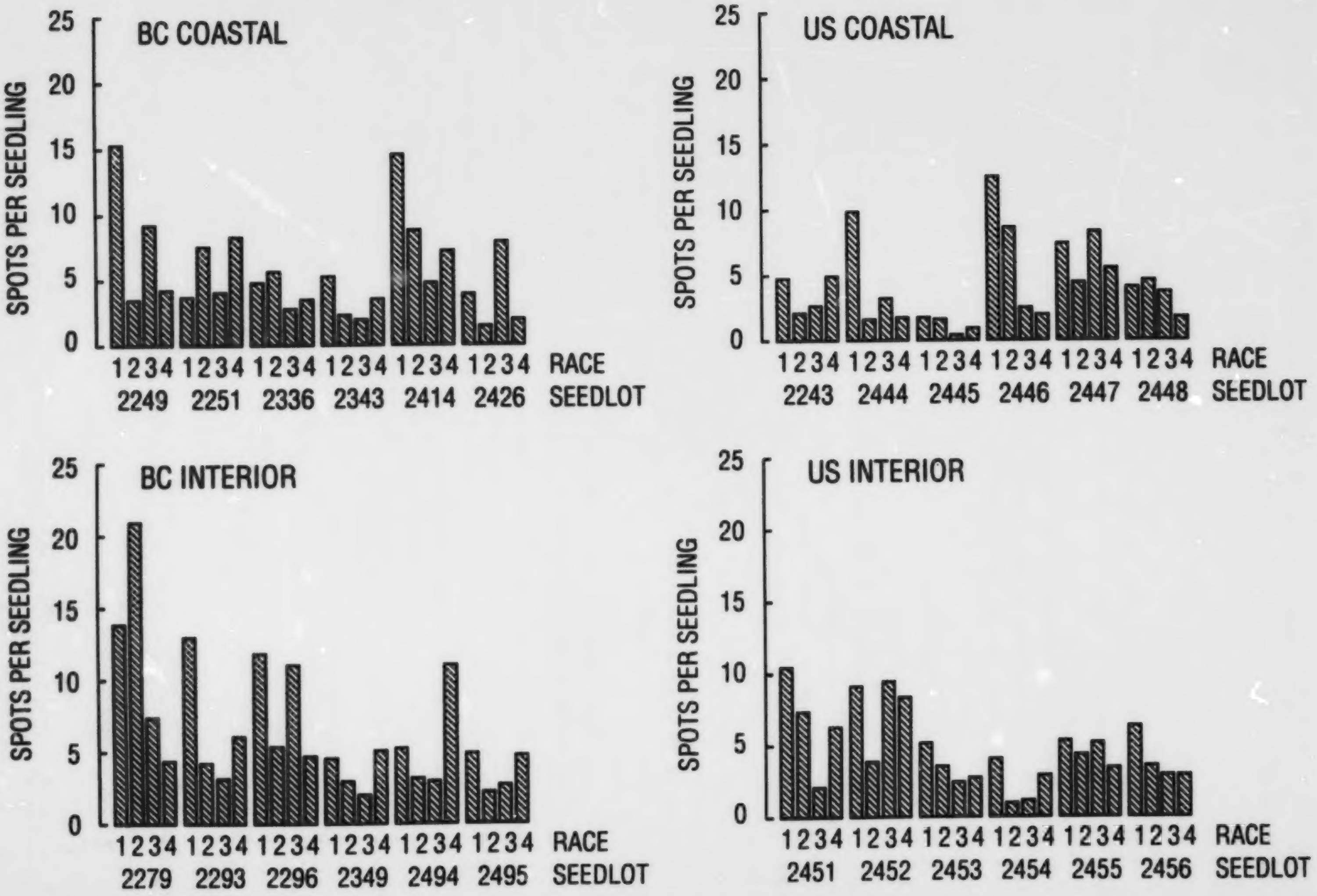

Figure 4. **Mean flecks + spot per tree per rust source** (race) **by seed parent** (seedlot) **grouped by region of origin (e.g., B.C. coastal).** Race 1 = coastal mix (Whistler), Race 2 = Nakusp, Race 3 = Valemount, Race 4 = Cowichan.

2) on a nearby source (Mount Revelstoke E; seedlot 2279), while the lowest mean value (0.54 spots) came on seedlings from a Mount Hood, Oregon, parent (seedlot 2445) inoculated by the Valemount rust (no. 3).

Analysis of variance (Table 2), based on replicate means, indicated significant effects of rust source, parent-tree country and zone (e.g., coastal vs. interior), and parent tree. However, the race × country × zone × seedlot interaction term was significant, indicating variability in results among parents from a region against these rust sources.

Further analysis of spot frequency will entail covariate adjustment of spore density, spots level in seedling rack during inoculation, and seedling size before determining the importance of the main effects on rust-infection success and progress.

DISCUSSION

The lack of correlation between rust spore counts and mean spots per tree may be due in part to imprecision in defining infections as spots. Some big spots may have been a few small spots combined. Also, it is likely that not all spores were viable or possible that high spore density impeded spore germination. Seedlots from the U.S. were selected to be infectable (develop spots), while possessing known or hypothesized resistance mechanisms; since generally they developed fewer spots than the untested B.C. families, the trend obtained is that expected. However, within each country, seedlots from the interior regions generally developed more spots than their coastal counterparts, especially when exposed to the coastal inoculum sources. More distinct regional differences may appear from subsequent examinations when the defense mechanisms, such as needle shed and bark reactions, can take effect.

The preponderance of yellowish spot colors on seedlings from the inland seed sources (interior B.C. and U.S.) exposed to coastal rusts, rather than the more balanced pattern in coastal seed

Table 2. Summaries of analyses of variance of spots plus flecks (all spots) **and spots only**

Variance source	df	Probability	
		All spots	Spots only
Country (Co)	1	***[a]	***
Zone (Zo)	1	***	***
Co × Zo	1	0.331	0.888
Seedlot/Zone (SL)	20	***	***
Race (source)	3	***	***
Race × Co	3	0.708	0.453
Race × Zo	3	0.094	0.004
Race × Co × Zo	3	0.151	0.985
Race × Co × Zo × SL	60	***	***
Replicate (Rep)	9	***	***
Co × Zo × SL × Rep	207	***	***

[a] *** = probability < 0.001.

sources, suggests a physiological difference between the coastal and inland white pine populations, perhaps making the inland trees more susceptible to rust genotypes producing yellow spots (McDonald and Hoff 1975). Conversely, the gene frequencies in the coastal and inland rust populations may differ. In view of the evidence of limited genetic variation in white pine over the same portion of its range (Steinhoff et al. 1983; Rehfeldt et al. 1984), the importance of the difference in spot color will be assessed carefully.

SUMMARY

Inoculation success of 24 common seed parents was adequate for all rust sources. Statistical analyses indicated important differences among rust source (race), origin of seed parent (region), and parent tree within region, but little effect of rust spore density. Interaction terms involving these sources of error were significant, indicating complex, rather than simple, differences in host-pathogen relationships. Coastal rust sources seem more virulent on interior seedlings than do interior rusts.

ACKNOWLEDGMENTS

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RESISTANCE TO FUSIFORM RUST IN SOUTHERN PINES: HOW IS IT INHERITED?

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INTRODUCTION

It has been acknowledged for decades that fusiform rust (caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*) is the most destructive disease of slash and loblolly pines in the southern forests and that resistance is the only feasible control. Yet, little is known about how resistance is inherited. Most often, polygenic inheritance is assumed (Powers et al. 1981), possibly because the observed distribution of phenotypes along some index of susceptibility (usually, the proportion of galled seedlings in a family or number of galls per tree) appears to be continuous. Since the kinds of experimental materials most frequently used are either open-pollinated families or inocula bulked from different sources (or both), results can only be analyzed and interpreted in relative terms or by quantitative genetic parameters (e.g., heritability, general and specific combining ability). In this way, the expectation of quantitative inheritance can become self-fulfilling, but deceptive. Continuous distribution does not necessarily imply polygenic inheritance; segregation at even a single locus can sometimes mimic quantitative inheritance, especially if environmental variance is involved (Thompson 1975). In a wild pathosystem with only a few major interacting genes for resistance/virulence at intermediate frequencies, the expected distribution is an approximately continuous array of phenotypes.

In many pathosystems that have been carefully studied (Person 1959; Powers 1960), major genes for resistance in hosts (usually dominant) interact specifically with complementary genes for avirulence in pathogens to condition a resistant (incompatible, low) infection type. When an alternate allele for virulence at the same gene locus in the pathogen is substituted, the interaction changes to a susceptible (compatible, high) infection type. Resistance and virulence are thus not intrinsic properties of hosts and pathogens; their expression depends on the genotype of the other symbiont. Only the *interaction* is inherited, and this can be determined only when precise genetic control is exercised over both host and pathogen. This unique feature of interorganismal genetics (Loegering 1984) is one that has been largely ignored or misunderstood in the literature on fusiform rust. Quantitative genetic methods are ill-suited for dealing with genetic interaction at this level, and conclusions based on these methods can be inaccurate or misleading. For example, polygenic inheritance is often equated with horizontal resistance which, by definition, is stable to racial variation in the pathogen. If the observed resistance is in fact specific, the assumed stability will vanish as soon as the frequency of the matching gene for virulence in the pathogen population increases.

Here, we consider a case in point: an experiment designed to examine the inheritance of fusiform rust resistance, in which both pine hosts and rust inocula were genetically controlled with relative precision (selfed and full-sib host families and inocula derived from single galls--the only case of its kind we are aware of) but in which results were interpreted quantitatively (Griggs and Walkinshaw 1982). In our reanalysis of this data, based on biological properties inherent in gene-for-gene systems, we find evidence for two specific interacting loci in host and rust, and we report new data describing the mechanism of resistance of one of the genes.

MATERIALS AND METHODS

Griggs and Walkinshaw (1982) challenged an array of full-sib and selfed families of slash pine (*Pinus elliottii* Engelm. var. *elliottii*) from a five-parent complete diallel crossing plan, with two single gall isolates and one composite inoculum of fusiform rust. Parents were selected for extremes of resistance or susceptibility, based on other tests, as were the single-gall inocula for relative virulence and avirulence; one of these was isolated in the field from an offspring of one of the resistant parents. Seedlings were inoculated individually using the forced-air apparatus of Snow and Kais (1972) with an inoculum density of 12-18 spores/mm^2 directed against the succulent growing tip. Up to 48 six-week-old seedlings in available progenies (including reciprocals) were challenged with each inoculum and observed for typical gall development 9 months later. Several crosses did not take well, resulting in missing cells with one or more of the inoculum/family combinations. Further details are in Griggs and Walkinshaw (1982).

To investigate mechanisms of resistance, different half-sib seedlings of one of the resistant parents were challenged with the two single-gall inocula and examined histologically. Seedlings (24 for each inoculum) were harvested 30 days after inoculation and the target (symptomatic) area excised, embedded in paraffin, and stained, using standard techniques described earlier (Walkinshaw 1978).

RESULTS AND DISCUSSION

Inheritance of Resistance

Since reciprocal crosses in the data of Griggs and Walkinshaw (1982) showed no appreciable differences from one another in their response to the different inocula (with one exception, discussed below), we show the pooled data in Table 1.

The most striking result is the difference in performance of progenies of parent 8-7 to the two single-gall inocula; almost none were infected by LM-5 and almost all were infected by 8-7-8. These clearly qualitative differences suggest the interaction of major genes. Since there is no segregation in any of the full-sib or selfed families of parent 8-7 in interaction with inoculum LM-5, it appears that this tree is homozygous dominant for resistance (R_1R_1). The lack of resistance of 8-7 families to inoculum 8-7-8 strongly suggests that this isolate has the matching gene (v_1) for virulence to R_1. An apparent inconsistency is the cross between 8-7 and 18-26, which averaged 12.5% infection. When 8-7 was used as the pollen parent, this family was 25% infected; but when used as seed parent, it had no infection, suggesting that pollen contamination may have been the cause of the discrepancy. This rationale is supported by the absence of infection on any of 8-7's open-pollinated progeny.

The other interesting parent in this matrix is 18-27. There are only two cells in the matrix for this parent against LM-5--one in a cross with 8-7 and the other with 18-26. Progeny of the latter are close to a 1:1 ratio of resistant:susceptible (45% infected, Table 1), suggesting segregation at a locus

Table 1. Percent infection of slash pine seedlings from full-sib, self, and open-pollinated families inoculated by basidiospores derived from single-gall and bulk isolates of fusiform rust (adapted from Griggs and Walkinshaw 1982[a])

Inoculum	Parents	8-7	18-27	9-2	18-26	18-62	Wind
LM-5	8-7	0	0	0	12.5[b]	0	0
	18-27		-	-	45	-	46
	9-2			-	-	-	25
	18-26				88	91	88
8-7-8	8-7	95	54.5	98	92	88	100
	18-27		50[c]	-	46	69	46
	9-2			-	-	-	92
	18-26				88	92	96
Bulk	8-7	50	29	58	67	67	62
	18-27		25	25	67	33	54
	9-2			72	73	75	71
	18-26				92	96	88

[a] From a diallel mating plan: data from reciprocal crosses pooled.

[b,c] Significantly different from 1:0 and 3:1 ratios expected, respectively.

heterozygous for resistance in 18-27. Clearly, it cannot be the same allele as in 8-7 because relatively high resistance is maintained in all progenies to the other isolate, 8-7-8, which overwhelms all offspring of 8-7 except when 18-27 is the other parent. In fact, most full-sib families of 18-27 segregate in a 1:1 ratio to both inocula; average infection of all but one of the full-sib and open-pollinated families was 51%. (No segregation is apparent in offspring of 18-27 × 8-7 against inoculum LM-5 because expression of any other gene would be masked by R_1.) The selfed family of 18-27 is a discrepancy; a 3:1 ratio of resistant to susceptible is expected in a selfed heterozygote, but a 1:1 ratio (50% infection) was observed. This inconsistency apart, we can tentatively assign the gene to another locus, R_2, and designate the parent genotype R_2r_2 (although the possibility of an alternate dominant allele at locus 1 cannot be excluded). No complementary allele for virulence to R_2 (i.e., v_2) is evident in the data for any of the inocula, and its existence is hypothetical.

Since the cross 18-26 × 18-62 produces offspring that are highly susceptible to both inocula, both parents appear to be homozygous recessive at both loci (r_1r_1 r_2r_2). Nothing firm can be said about parent 9-2 because most of the cells are missing in the single-gall inocula.

We tested the hypothesis of two independent loci for resistance to the two inocula on each full-sib family in the single-gall inoculations (except those with 9-2 as a parent) by chi-square. One of seven families inoculated with LM-5 had a significant value (8-7 × 18-26, the cross suspected of pollen contamination), as did one of nine inoculated with 8-7-8 (18-27 × self). The last family had only 14 seedlings and was more liable to sampling error. All of the open-pollinated families (not including 9-2) inoculated with single-gall isolates were consistent with the hypothesis being tested.

The inference of two genes for virulence at independent loci in the rust is based on the complementarity that exists in gene-for-gene relationships. Proof would require Mendelian segregation (1:1 ratio) of compatible:incompatible reactions induced by single, haploid basidiospores on a host genotype known to have the appropriate resistance allele. This would be nearly impossible to observe with the inoculation technique used in these tests, in which spores were impacted at high densities on small target areas. Isolating and applying single spores to infection courts (succulent shoots) of limited area on young seedlings would be difficult under any circumstance, and we know of no successful attempts. With inoculation techniques currently used, it seems likely that in any inoculum heterozygous and segregating for virulence/avirulence, virulent spore segregants will almost certainly mask expression of avirulent spores at high inoculum densities and small target areas commonly obtained. Heterozygous inocula will thus appear and behave as though homozygous. The uniform avirulence of LM-5 on all progenies of slash parent 8-7 and the nearly uniform virulence of 8-7-8 on progenies of the same parent (except when 18-27 is the other parent) are consistent with this rationale and strongly suggest that these single-gall inocula are fixed at the two loci in question.

The futility of using composite inoculum in tests of inheritance is evident in the last third of Table 1. The full-sib families range from 25 to 96% infected, half-sib family means from 54 to 79%, and open-pollinated families from 54 to 88% more or less continuously in each case. The winners and losers can be picked at some arbitrary cut-off point, but no genetic hypotheses can be tested because frequencies of genes for virulence/avirulence in the inoculum mix are unknown. With hindsight gained from the single-gall inocula, we can tentatively suggest that the frequency of v_1 is relatively high in the bulked inoculum (since 8-7 progeny have intermediate levels of infection), and that the hypothetical v_2 is still absent. It is unfortunate that seeds, scarce or lacking in some crosses, were prioritized for this test at the expense of the tests with single-gall inocula.

The genetic interactions hypothesized above may be visualized more clearly by comparing a model of a two-locus gene-for-gene system with the observed data. In the model (Fig. 1), the four possible haploid genotypes of the rust are arrayed against the four diploid genotypes of the pine host relevant to loci expressing dominance. The 16-cell matrix shows the symmetry and geometrical progression characteristic of gene-for-gene systems, in which addition of a resistance allele to a genotype halves the number of races of the pathogen capable of attacking it (see Person 1959 or Robinson 1987 for more detailed illustration and analysis). Resistant (incompatible) interactions only occur in cells where a dominant R allele is *not* matched by a complementary v allele.

In Figure 2, the four relevant pine genotypes (top line) are associated with the specific combinations of the parental crosses capable of producing them (last line). Redundance is present in crosses producing genotypes R_1- r_2r_2 and r_1r_1 R_2- since both 18-26 and 18-62 donate recessive alleles at both loci. For example, all seedlings from the cross 8-7 (R_1R_1 r_2r_2) × 18-27 (r_1r_1 R_2r_2) will receive an R_1 allele from 8-7 and an r_1 allele from 18-27 and express complete resistance to LM-5 (av_1 av_2). But only half the offspring of this cross will get an R_2 allele from 18-27 and also be resistant to inoculum 8-7-8 (v_1 av_2); the other half will get r_2 from both parents. Consequently, only half the offspring will be heterozygous for both dominant alleles.

Genotypes: Pine

Rust

	$r_1r_1\ r_2r_2$	$R_1\text{-}\ r_2r_2$	$r_1r_1\ R_2\text{-}$	$R_1\text{-}\ R_2\text{-}$
$av_1\ av_2$	+	-	-	-
$v_1\ av_2$	+	+	-	-
$av_1\ v_2$	+	-	+	-
$v_1\ v_2$	+	+	+	+

Figure 1. Genetic interactions in a two-locus gene-for-gene pathosystem. Allele symbols R and r stand for resistance and susceptibility in hosts av and v for avirulence and virulence in pathogens, at the loci indicated by subscripts.

Genotypes: Pine

% infection (observed/expected)

Rust

	$r_1r_1\ r_2r_2$	$R_1\text{-}\ r_2r_2$	$r_1r_1\ R_2\text{-}$	$R_1\text{-}\ R_2\text{-}$
$av_1\ av_2$ (LM-5)	91/100	6/0	45/50	0/0
$v_1\ av_2$ (8-7-8)	92/100	93/100	50/75	55/50
$av_1\ v_2$	+	-	+	-
$v_1\ v_2$	+	+	+	+
Parent combination:	**18-26 x 18-62**	**8-7 x 18-26 18-62**	**18-27 x 18-26 18-62**	**8-7 x 18-27**

Figure 2. Genetic interactions among putative slash pine-fusiform rust genotypes. Pine genotypes are diploid and inferred from the data in Table 1. Here, data are pooled over genotypes and denote the percent infection (observed/expected) for the host-rust interaction indicated. Rust genotypes represent haploid basidiospores derived from dikaryotic aeciospore inocula assumed to be homozygous at the two hypothesized loci. The + and – indicate compatible and incompatible interactions predicted by the model.

Since neither inoculum is virulent to R_2, v_2 is not present in either inoculum, leaving half of the matrix in Figure 2 vacant and hypothetical. Of the eight full cells, none deviate significantly from expectation (again excepting the cell R_1- r_2r_2/av_1 av_2, which has a minor deviation perhaps due to pollen contamination).

Mechanism of Resistance

Histological interactions between fusiform rust and pine hosts range from no visible symptoms, through different degrees of incompatibility, to normal gall development (Miller et al. 1976). In compatible infections, the fungus penetrates the epidermis of succulent shoots, establishes in cortical parenchyma tissue, then ramifies through ray parenchyma--often all the way to the pith. Haustoria are common and necrosis uncommon in early stages of disease development. A typical example of a compatible reaction 30 days after inoculation, between an offspring of parent 8-7 (R_1) infected by virulent inoculum 8-7-8 (v_1), is shown in Figure 3a. A contrasting incompatible reaction, typical for offspring of this same parent and inoculum LM-5, was a kind of hypersensitivity (Fig. 3b). In these instances, cells surrounding the zone of initial infection rapidly became necrotic, and haustoria, if present at all, were abnormal--often shriveled or encrusted. A necrophylactic periderm walled off the entire infection zone.

Table 2 quantifies our observations on 24 half-sibs of parent 8-7 challenged with each inoculum. These data were reasonably consistent with the infection data in Table 1. Superficial stem lesions (purple spots) were common to both compatible and incompatible reactions and so were not early predictors of the final outcome. Periderm formation was not found in 11 of the incompatible interactions, but could have escaped observation; often in hypersensitive reactions, only a few cells are affected. The histological evidence most consistent with the final symptom data was the complete absence of viable mycelium in incompatible combinations. Mycelium was present in most compatible combinations, and the instances in which it was not could have resulted from escape of infection or observation. Unfortunately, we have no observations of progenies of parent 18-27 except those confounded with 8-7.

Miller et al. (1976) observed resistant reactions identical to the ones depicted in Figure 3b on slash pine seedlings of unidentified pedigree, as well as cases of more pronounced and rapid hypersensitivity involving only a few cells. They also reported intermediate degrees of incompatibility/compatibility wherein larger volumes of tissue were infected before being isolated by a periderm, and still others in which gall formation was restricted. These observations are reminiscent of True's (1938) partially resistant reactions of Scots pine (*P. sylvestris* L.) against western gall rust (*Peridermium harknessii* Moore), where breach of periderm barriers by rust mycelium was followed by formation of sequent periderms by the host in a continuing, dynamic struggle between host and fungus. In this continuum of reaction types, the outcome seems to be determined not by the *kind* of reaction but rather the *rate* of reaction that is induced in the host (Kinloch 1972).

Jewell (1966) was the first to propose that resistance to fusiform rust in slash pine is controlled by a dominant gene. He based his hypothesis on segregation data of full-sib seedlings of two of the same parents discussed here, 8-7 and 18-27. At the time, he considered that the same gene was present in both parents but later rejected his hypothesis when subsequent inoculations revealed inconsistencies in the data (Jewell and Mallett 1967). In both cases, however, the inoculum used was from uncontrolled, bulk lots. Our data, which is based on stricter genetic control of both host and rust, suggest that Jewell's (1966) hypothesis was correct, except that each parent has a different gene that interacts specifically with a complementary gene in the pathogen. We cannot prove this hypothesis with the data available because formal proof of a gene-for-gene relationship requires demonstration of Mendelian ratios in controlled crosses of pathogen as well as host (difficult, at best, with a rust of a woody perennial) and

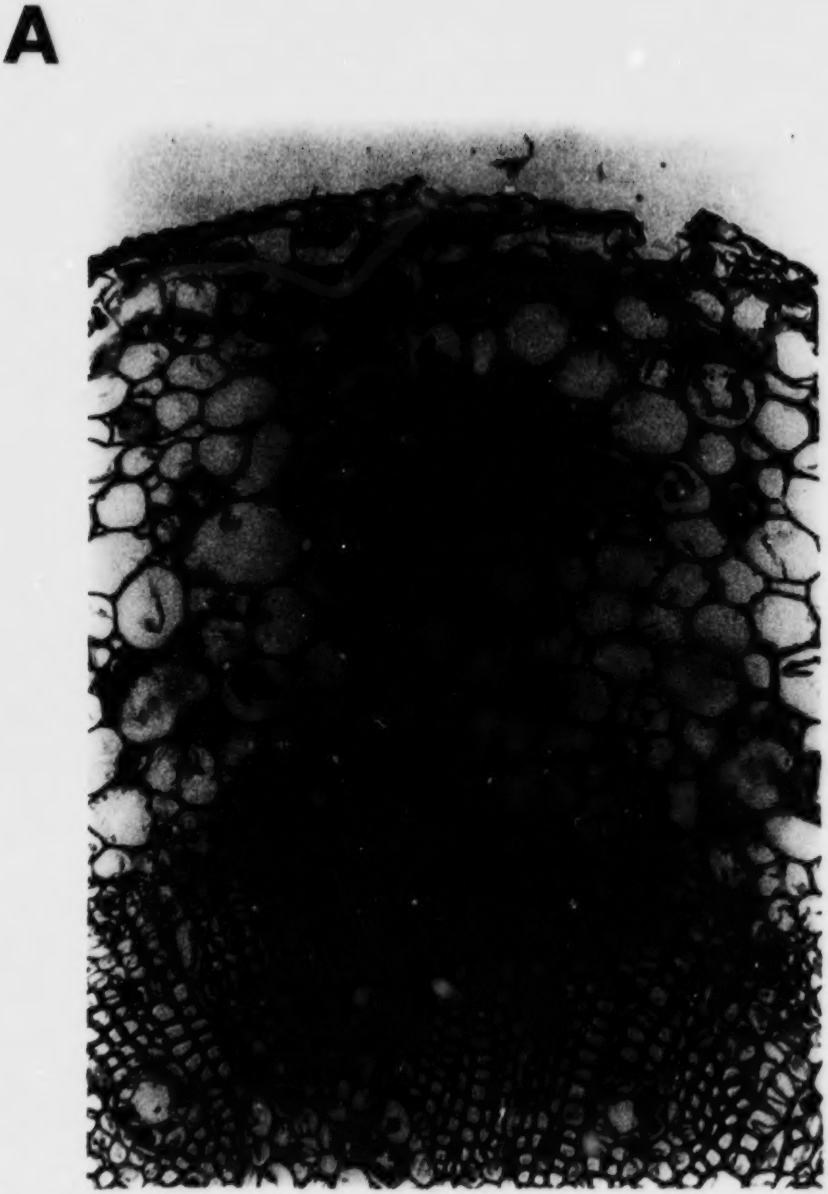

Figure 3. Histological reactions of slash pine seedlings (both half-sibs of parent 8-7) to two different isolates of fusiform rust 30 days after inoculation. a. Compatible (susceptible) reaction to inoculum 8-7-8. Note deeply staining reaction zone (RZ) with mycelium (m) and occasional haustoria (h) extending to pith. **b.** (See next page.)

Figure 3b. Incompatible (resistant) reaction to inoculum LM-5. Note zone of collapsed, necrotic cells isolated by a periderm (p).

Table 2. Histological responses of slash pine seedlings from parent 8-7 to avirulent (LM-5) and virulent (8-7-8) inocula

Inoculum (putative genotype)	No. seedlings					
	Surface lesion		Periderm formation		Mycelium	
	+[a]	–[b]	+	–	+	–
LM-5 (av_1)	14	10	13	11	0	24
8-7 (v_1)	17	7	4	19	17	7

[a] + = presence.

[b] – = absence.

because our data are incomplete (Table 1, Fig. 2). If, however, an isolate were found that was avirulent to progeny of 8-7 and completely virulent to progeny of 18-27 (i.e., fit the model av_1 v_2; cf. Fig. 2 line 3), an alternative interpretation would be difficult to defend. Of course, additional loci for resistance/avirulence than the ones we hypothesize here may also exist in this pathosystem.

We realize that gene-for-gene interactions do not preclude the modifying influence of other nonspecifically acting genes in the final outcome of the host-pathogen encounter. But major genes have major effects, and identification of these genes is more useful for describing and predicting the dynamics of a pathosystem than are quantitative genetic parameters for the host alone. With knowledge of specifically interacting genes and their frequencies, strategic deployment of major host genes can be made and the epidemiological consequences predicted.

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FAMILY PERFORMANCE OF FUSIFORM RUST RESISTANT LOBLOLLY PINES IN A SEEDLING SEED ORCHARD[1]

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ABSTRACT

A loblolly pine seedling seed orchard (SSO) established with rust-free survivors from the concentrated basidiospore spray (CBS) system of inoculation has begun producing very resistant progeny. The proportion of SSO trees within families that produced resistant progeny varied from 14 to 100%. All 55 trees in four families produced resistant progeny as indicated by the CBS inoculations. Seven families had 75-93% of the SSO trees producing resistant progeny. For the 24 families in this study, neither the initial infection after CBS inoculation nor the amount of rust on survivors in the SSO indicated the resistance of progeny of SSO trees. The SSO method has helped to identify trees with highly resistant progeny. Trees with highly resistant progeny have disease ratios of less than 0.30. The intensive screening for rust resistance in the SSO has enabled us to incorporate unique genes for resistance in trees with good silvicultural characteristics.

INTRODUCTION

The best means of limiting damage by fusiform rust (caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*) in young southern pine plantations appears to be by planting resistant seedlings. Rust-resistant loblolly pine (*Pinus taeda* L.) seed orchards are being developed cooperatively by the USDA Forest Service and the Georgia Forestry Commission. Both clonal and seedling seed orchards (SSO) have been established. SSOs were established in order to increase heterogeneity in the rust resistant material.

Powers and Kraus (1983) established a rigorous procedure for rust resistance selection for the SSO. Loblolly pine seedlings are inoculated by the Concentrated Basidiospore Spray (CBS) system (Matthews and Rowan 1972). Our standard measure of familial resistance is the disease ratio (DR), computed by dividing the percent of seedlings with galls in the test family by that of the standard susceptible control. Test families with a DR > 0.70 are considered susceptible, those with a DR ≤ 0.70 are resistant (Kuhlman and Powers 1988). Seedlings with galls in resistant families are culled. Healthy survivors from 14 to 16 resistant families are planted in orchard blocks at close spacings (1418 trees per ha). Survivors developing field infections are rogued. Other trees are rogued on the basis of slow growth or poor crown form. When an orchard tree produces seeds, those progeny are tested in the CBS system. Seed-producing trees are rogued by DR to a final density of 119 trees per ha. In all, 92% of the original trees are rogued.

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[1] This work was supported in part by cooperative agreements with the Georgia Forestry Commission.

The SSO has several advantages over the more traditional clonal orchard. Foremost is the opportunity to diversify the genetic base in the orchard with a heterogeneous seedling population. Second, we can identify highly resistant trees. Third, unique resistance genes can be incorporated in trees with good silvicultural characteristics to save resistance genes that might otherwise be discarded. Finally, progeny from SSOs are more rust resistant than those from clonal orchards (Powers and Kraus 1986).

The disadvantages of the SSO system are the increased time and labor involved in establishing, maintaining, and evaluating each tree. Roguing sometimes creates excessively large empty spaces in some places and leaves too many good trees in others. Finally, certain of the original families may be disproportionately represented in the final stocking because of their high rust resistance or good growth characteristics.

In the last few years the number of SSO trees producing cones has increased dramatically. Last year we tested progeny of more than 400 trees. The labor and time involved has become considerable. We wondered if we could discern patterns of inheritance that would help us to reduce our workload. The objectives of the present study were twofold. 1) What is the proportion of trees within families that produce resistant progeny? 2) Is this frequency related to initial screening or amount of rust in the orchard?

METHOD

Information presented in this paper is derived from SSO blocks established in 1975 and 1976. Second-generation progeny make up the SSO blocks. Those progeny were first screened in the CBS system and seedlings with galls were eliminated. The percentage of rust-free survivors of the CBS tests that developed galls during the first 4 years in the SSO was determined for each family. Progeny of SSO trees were tested in the CBS system, and if the DR ≤ 0.70, the SSO tree was rated as resistant. Diameter breast height (dbh) at age 13 was used to evaluate relative growth potential.

RESULTS AND DISCUSSION

Five or more SSO trees from 24 families (= first-generation sources) have been screened in the CBS system. The percentage of seedlings (second generation progeny) with galls in the CBS screening varied from 14% for family 3333 to 72% for family 7-56 (Table 1). Most families had 30-40% of seedlings with galls. Several families (SML-9, HH, and 10R) were very resistant to infection in the field (2, 2, and 4%, respectively), whereas families T601 and T605 had 44 and 45% infection, respectively. The correlation coefficient for CBS results and field performance of survivors in these families is r = 0.34. Previously, Powers and Kraus (1983) reported a good correlation (r = 0.755) for these traits. Their data set included both loblolly and slash pines. The last two columns in Table 1 present data on the number of SSO trees with progeny that have been tested and the percentage of those with a progeny DR ≤ 0.70. All SSO trees from four families (29R × 10-5, 42R, T605, and 3333) have produced resistant progeny. In the next group of seven families, 75-93% of the SSO trees have produced resistant progeny. The third group of eight families had 43-61% of the SSO trees producing resistant progeny. The final group of five families had only 14-36% of the SSO trees producing resistant progeny. We hypothesize that the differences in family responses are due to the number of genes involved in resistance in these families. Families in the 80-100% resistant progeny group probably inherit their resistance from a single gene. Those in the 40-60% resistant group may have two genes that must both be present to convey resistance. Those in the 20-36% resistant group probably have several resistance

Table 1. **Performance of second- (SSO) and third-generation progeny from 24 families or first-generation sources**

Family	CBS galls (%)	Galls in SSO (%)	SSO trees tested	
			Number	% with resistant progeny[a]
29R × 10-5	22	16	25	100
42R	41	18	19	100
T605	38	45	6	100
3333	14	15	5	100
11-20	36	14	28	93
10-5	40	18	36	92
2318	44	32	13	92
11-9	40	13	11	91
29R	41	38	17	82
T601	43	44	15	80
10R	38	4	8	75
15-42	49	21	18	61
1582-11 × 1590-6	31	12	10	60
29R × 1495-35	22	22	23	57
TFS[b]	38	20	14	57
1495-35	29	21	11	55
10-6	37	8	19	53
SML-9	34	2	9	44
7-56	72	25	7	43
LP[b]	57	21	11	36
1495-18	68	26	7	29
TDR[b]	37	10	10	20
3312	50	17	6	17
HH[b]	31	2	7	14

[a] Progeny designated as resistant if DR ≤ 0.70.

[b] Composite seed collections from geographic sources (TFS = Texas Forest Service; LP = Livingston Parish, Louisiana; TDR = Texas Drought Resistant) or HH = Hitchiti Hybrids (shortleaf × loblolly hybrids).

genes that may be on different chromosomes providing few opportunities for the progeny to inherit all of the resistance genes.

We could use this information to eliminate testing of SSO trees from families with 80-100% probability of having resistant progeny, while we continue to screen trees in the lower categories. The relative resistance of individual trees within these families must also be considered, however. Many times we use the relative resistance of SSO trees to decide which trees to retain in the orchard and which ones to rogue.

Three resistance categories were established: susceptible with a disease ratio > 0.70; resistant with a disease ratio from 0.30 to 0.70, and highly resistant for disease ratios < 0.30 (Table 2).

From the cross of 29R × 10-5, 25 SSO trees were tested; 12 of those produced highly resistant progeny that averaged 15% of seedlings with galls. Thirteen trees produced resistant progeny that averaged 42% with galls. We believe that highly resistant SSO trees have inherited different resistance genes from each parent, whereas resistant trees have resistance gene(s) from only one parent (Kuhlman 1989).

Family 10-5 produced 6 trees in the highly resistant category, 27 trees in the resistant category, and 3 in the susceptible category. One of the highly resistant trees averaged only 3% of the seedlings with galls in several different tests with composite inoculum (Kuhlman and Powers 1988; Kuhlman 1989). Other families less frequently produced trees with highly resistant progeny, but we feel that identifying these highly resistant SSO trees justifies continued screening.

One of the benefits of the SSO approach to developing a resistant orchard is the potential to save unique resistance genes by selecting SSO trees with unique resistance and good silvicultural characteristics. Resistance in family 11-20 is unique. Kuhlman (1989) inoculated seedlings of 11-20 and 19 other resistant loblolly progeny with a rust isolate virulent towards 11-20. Family 11-20 was the only family as susceptible to that specific isolate as the susceptible control family. Although family 11-20 has also shown good resistance in field progeny tests, a tree improvement cooperative suggested its cooperators eliminate it from their rust resistance orchards because of its below-average growth characteristics. The SSO method enables the selection of individuals with good growth characteristics. Progeny of 11-20 are present in three blocks (151, 152, and 153) in our SSO as indicated by the first three digits in the tree number (Table 3). These blocks are the same age, but 151 and 152 were planted in January and 153 was planted in April.

Twelve trees from family 11-20 are still present in the orchard. For these 12 trees, Table 3 indicates how data from rust resistance of progeny can be combined with growth measurements to evaluate the relative merits of trees within families. Tree 153-362 is in our highly resistant group. It also has good diameter growth compared to the other two trees from this family in this orchard block. Similarly, tree 152-407 has a highly resistant disease ratio and good growth. Tree 152-196 has a disease ratio like the parent and the best growth of the 12. Thus, there are several SSO trees with resistance from 11-20 and with good growth potential.

In conclusion, the proportion of trees within families that produce resistant trees is dependent on the family. This proportion was not related to the initial disease ratio in the CBS inoculations, nor was it related to the amount of rust on the healthy survivors planted in the orchard. Testing the progeny of SSO trees has enabled us to identify highly resistant trees within some families. Furthermore, combining

Table 2. **The relative resistance of seedling seed orchard trees from 10 families indicated by resistance of their progeny in CBS tests**

Family	Highly resistant ($DR \le 0.30$)	Resistant ($DR > 0.30 \le 0.70$)	Susceptible ($DR > 0.70$)
29R × 10-5	12 (15%)[a]	13 (42%)	--
10-5	6 (13%)	27 (38%)	3 (81%)
29R	1 (22%)	13 (48%)	3 (76%)
3333	4 (10%)	1 (34%)	--
42R	1 (11%)	18 (40%)	--
11-20	2 (14%)	24 (39%)	2 (71%)
2318	--	12 (41%)	1 (75%)
T605	--	6 (42%)	--
T601	--	12 (41%)	3 (59%)
10-6	1 (26%)	9 (53%)	9 (61%)

[a] Number of trees in category (average percentage seedlings with galls).

Table 3. **Rust susceptibility and diameter breast height (dbh) of 12 trees from family 11-20 in three blocks of the seedling seed orchard**

Tree	Disease ratio (DR) of progeny	Dbh[a]
153-362	0.14	28.2
153-378	--	26.4
153-510	--	20.8
152-407	0.25	32.0
152-122	0.34	29.7
152-196	0.41	32.8
152-329	0.43	32.0
151-26	0.32	25.1
151-654	0.37	24.9
151-620	0.47	28.2
151-144	0.55	27.2
151-183	0.63	30.0

[a] Age 13, dbh in cm.

the rust data with growth characteristics lets us incorporate unique resistance genes into trees with good growth traits.

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EPIDEMIOLOGIC FUNCTION OF BLISTER RUST RESISTANCE: A SYSTEM FOR INTEGRATED MANAGEMENT

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Since the introduction of *Cronartium ribicola* J.C. Fisch. into western North America (Hoff 1988), forest managers have been searching for ways to combat the fungus (Hagle et al. 1989). Many avenues, such as elimination of the alternate host (*Ribes* spp.) and direct attack with fungicides, were tried. All failed when used by themselves (Ketcham et al. 1968). The ultimate method was believed to be resistance breeding. A program was initiated in 1950 to develop resistant populations of western white pine (*Pinus monticola* Dougl.), for the forests of northern Idaho, western Montana, and eastern Washington (McDonald and Hoff, these proceedings). By 1972, resistant populations were available and commercial planting of resistant stocks began (Gerhold et al. 1986). However, one serious problem remained. The available material was of somewhat restricted genetic breadth and was at considerable risk to racial variation in the rust (Kinloch and Comstock 1980; Yokota 1983; McDonald et al. 1984) as well as risk of ecophysiological maladaptation and susceptibility to other pests.

One way to rapidly expand genetic breadth in the pine population is to develop an integrated rust-management plan that would allow use of pine populations containing low to moderate levels of resistance (McDonald and Hoff 1982). This could be accomplished by measuring rust hazard (McDonald and Hoff 1982; Goddard et al. 1985) and then knowing how various control options would work on a given pine population growing where there was a specific degree of hazard (Hagle et al. 1989).

The integrated approach required a vehicle to bring to bear all the relevant information by individual site. An epidemiological model is proposed in this paper to incorporate both the hazard rating and prediction of performance of natural stands and plantations. The objective of this system is to predict performance of stock types in a variable environment by quantifying hazard and epidemiologic function of resistance.

A thorough knowledge of the biology of the disease had been accumulated through about 80 years of research (McDonald et al. 1981). Since the blister rust epidemiologic system contains an obligate alternate host; it represents two interlocking diseases. Three separate models (RIBRUST, RUSTMAN, and MTCLIM) were constructed or adapted to represent the disease (Fig. 1). RIBRUST represents the annual development of blister rust on the alternate host, four species of *Ribes* (*R. hudsonianum* Richards, *R. viscosissimum* Pursh, *R. inerme* Rydb., and *R. lacustre* (Pers.) Poir.). This model was developed as part of the initial blister rust simulator (McDonald et al. 1981). The major components of RIBRUST are site-specific weather and *Ribes* species-rust interaction (McDonald and Andrews 1981, 1982). The model accepts maximum and minimum daily temperatures and hourly

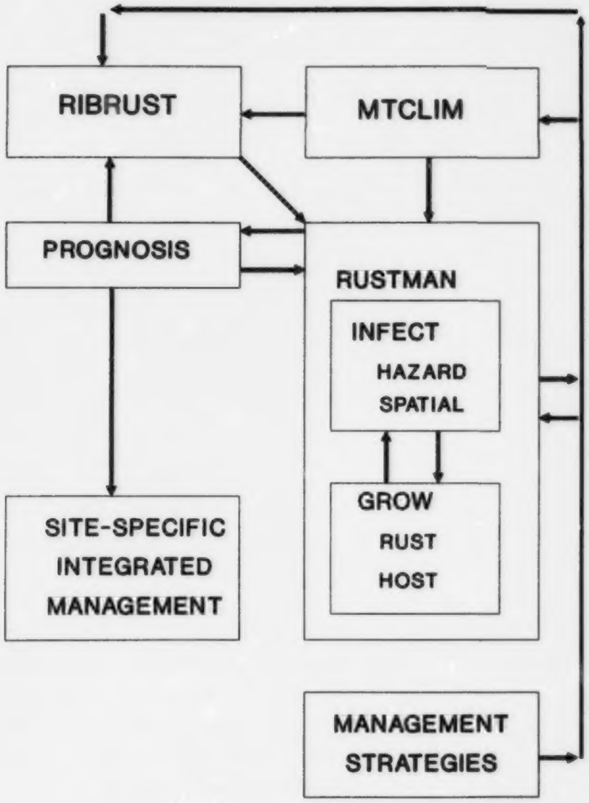
RIBRUST
MTCLIM
PROGNOSIS
RUSTMAN
INFECT
HAZARD
SPATIAL
GROW
RUST
HOST
SITE-SPECIFIC
INTEGRATED
MANAGEMENT
MANAGEMENT
STRATEGIES

Figure 1. Interaction of models comprising the proposed site-specific blister rust integrated management plan.

precipitation data to simulate annual urediniospore epidemics on the four *Ribes* species (Fig. 2) plus basidiospore concentration at individual pines as a function of *Ribes* and pine stocking density (Fig. 3). RIBRUST currently runs only on an IBM[1] mainframe computer. McDonald presently is exporting it to the IBM microcomputer environment. Extensive validation is necessary before it can be relied upon for management decisions.

RIBRUST and the pine infection model, RUSTMAN, can be driven by a correlative weather model MTCLIM (Running et al. 1987) or by actual weather data. If site-specific inputs from two regional weather stations, average annual precipitation isohyet, east and west horizon, elevation, slope, aspect, and leaf area index are available, MTCLIM will simulate hourly precipitation and temperature, daily minimum and maximum temperature, daily average and total radiation, and daylight average relative humidity.

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[1] Use of trade or firm names is for reader information and does not imply endorsement by the U.S. Department of Agriculture of any product or service.

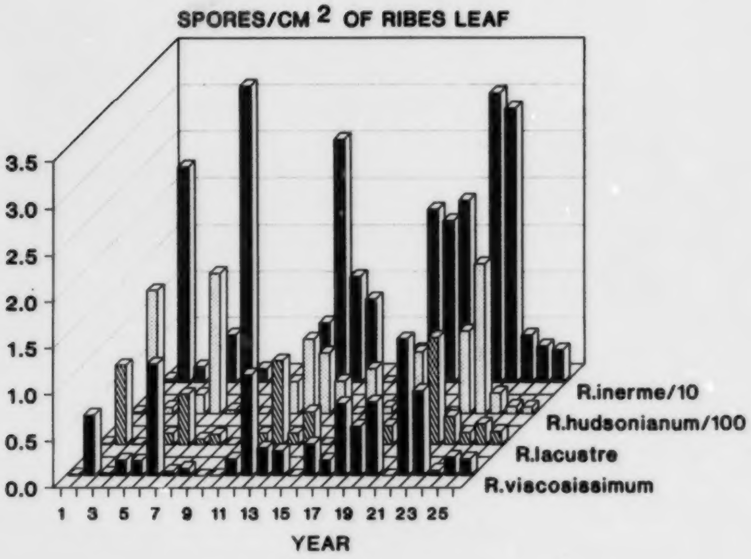

Figure 2. **Annual *C. ribicola* basidiospore output expectation as simulated by RIBRUST for four *Ribes* species from 26 consecutive years of weather records collected at Pierce, Idaho.**

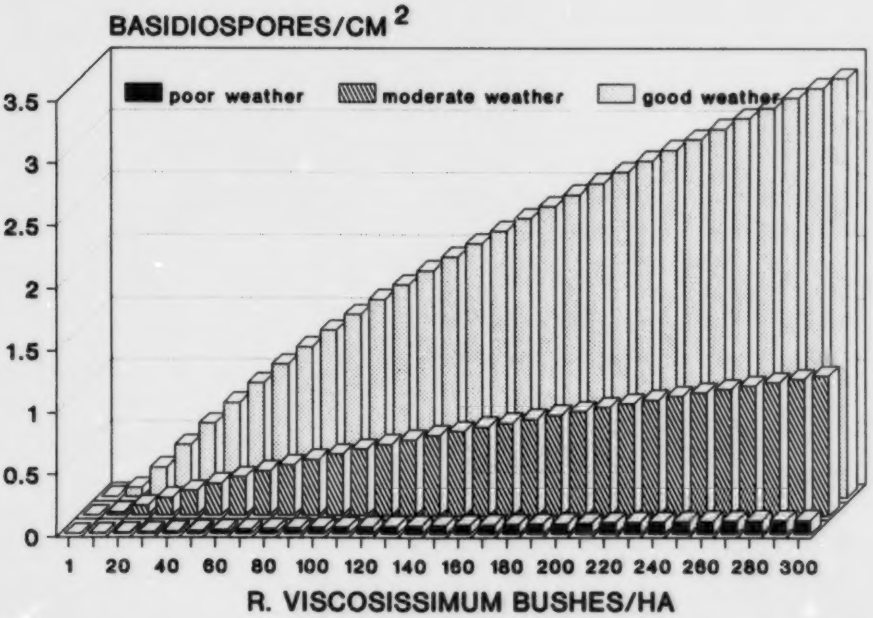

Figure 3. **Annual *C. ribicola* basidiospore density expectation as simulated by RIBRUST at *P. monticola* target for three growing seasons as a function of numbers of *R. viscosissimum* per hectare.** Weather records collected at Pierce, Idaho.

These climatic variables are then used to define the date and duration of infection periods, which are the required model inputs (McDonald et al. 1981).

RUSTMAN contains two separate models, INFECT and GROW. We will discuss INFECT, which is composed of a two-dimensional model of rust hazard (Fig. 1) and a three-dimensional model of spatial distribution of infections (multiple-infection equation). We will not discuss the functioning model, GROW[2], which deals with growth and development of individual infections on individual trees and includes facets to account for host growth rate, host size at infection, infection location, and host-rust genetic interaction. The entire RUSTMAN model will be part of version 6 of the forest growth simulator, the Stand PROGNOSIS Model (Stage 1973).

RUST INDEX: A MEASURE OF HAZARD

Rust hazard is quantified by counting the number of cankers accumulated on a tree and then dividing by the accumulated target that was exposed to infection (accumulated cankers per exposed target summed over exposure period) to calculate the rust index:

$$RI = \text{cankers/1000 needles/year} \qquad (1)$$

Of course, one cannot easily determine the accumulated target but number of new needles as a function of crown size (McDonald et al. 1981; Fig. 35) was used to compute the expected summed target of a tree that has grown in a defined fashion. The equations are

$$ST = \sum_{t=1}^{A} 2.6 \times H_t^{\left(H_t^{0.63} \cdot 1.32\right)} \qquad (2)$$

where

ST = summed target in thousands of needles
A = number of whorls or other age estimate in years
H_t = tree height in metres at age $t = GI/AP$

$AP = 0.114 \times [1 - 0.925 \times e^{(-0.020796 \times t)}]^{-2.488}$
and
$GI = H_t \times AP$ = index to individual tree growth.

The equation in AP is an adaptation of Brickell's (1970) site index equation for western white pine expressed in height in feet at 50 years.

In 1966 and 1967 the Northern Region of the Forest Service, U.S. Department of Agriculture, surveyed blister rust in 106 stands in northern Idaho for canker numbers, ages, and tree sizes. An 84-tree sample was measured and the stand age was determined[3]. From these data the average annual rust index was calculated by McDonald according to equation 1. The result for a single stand is shown (Fig. 4) and is typical of 35- to 50-year-old stands growing in northern Idaho. The reduction of rust index with age likely results from stand closure suppressing local *Ribes* populations (McDonald et al. 1981).

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[2] GROW is the part of RUSTMAN that simulates development of blister rust cankers within individual white pine crowns to the point of tree mortality. The manuscript describing GROW is in preparation by G.I. McDonald.

RUST INDEX: NATURAL INOCULATION

Rust index can be measured on trees growing in field locations under exposure to natural inoculation. This approach can be used to supply evidence for an epidemiologic parameter associated with various stock types. Rust index was calculated from data obtained at four locations in northern Idaho and northeastern Washington. Two plantations were the Priest River and Deception Creek vigor quality locations described previously (Steinhoff 1971; Goddard et al. 1985) and composed of six stock types. Seedlings were planted in family rows and the data presented in this paper were collected after 12 years. At least 100 tree samples of each stock type were measured for height, number of whorls, and number of cankers. One of the other two plantations, Merry Creek, has been described (Bingham et al. 1973) and the fourth, Gletty Creek, is a replication of Merry Creek. The latter plantations were composed of 1-acre blocks of four stock types: susceptible controls (CONT), general combiner F1 (F1GC), general combiner F2 (F2GC), and general combiner F1 backcrossed to their parents (F1GC × GC). At least 61 seedlings of each type were inspected in 1981 or 1982, after 11 or 12 years of exposure to natural inoculum. Number of whorls, height, and number of cankers were recorded.

RI was calculated using equation 1 for each of seven stock types growing in the four plantations (Fig. 5). Only controls and FIGC were common in all four plantings. The large variation of rust index over location and stock type was evened out by calculating relative rust index (*RRI*). Since comparable controls were used in all plantings, *RRI* was computed as the ratio of resistant index to control index (Fig. 6). The variation of *RRI* over stock type stabilizes into a pattern associated with stock type, as one would expect if the principal source of variation were resistance mechanisms that condition probability of infection (McDonald and Hoff 1975; Hoff and McDonald 1980).

RUST INDEX: ARTIFICIAL INOCULATION

Better understanding of the host-pathogen-environment disease triangle, cheaper and more rapid methods of measuring the epidemiologic parameters, and a quantitative method to make bare-ground estimates of *RI* were needed to make a system of integrated blister rust management realistic. If *RI* could be measured under controlled conditions, then one would have a method to measure *RI* for seedling populations. Toward this end, an inoculation method was developed that would provide natural delivery of basidiospores to targeted pines. The spores were to be delivered in the absence of free water but at a relative humidity of 100%. The distribution of spores to the target plants was to be random. The microclimate was to be uniform throughout the test location. A rotating inoculum bed-settling tower (Fig. 7) was constructed and tested to verify that basidiospore delivery pattern achieved the above goals[4]. We

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[3] Carlson, C.E.; Toko, H.V. 1966. Preliminary report: white pine blister rust incidence survey. U.S. Dep. Agric., For. Serv., State and Private For., Region 1, Missoula, Montana.

[4] The manuscript describing the device and spore delivery pattern is in preparation by G.I. McDonald.

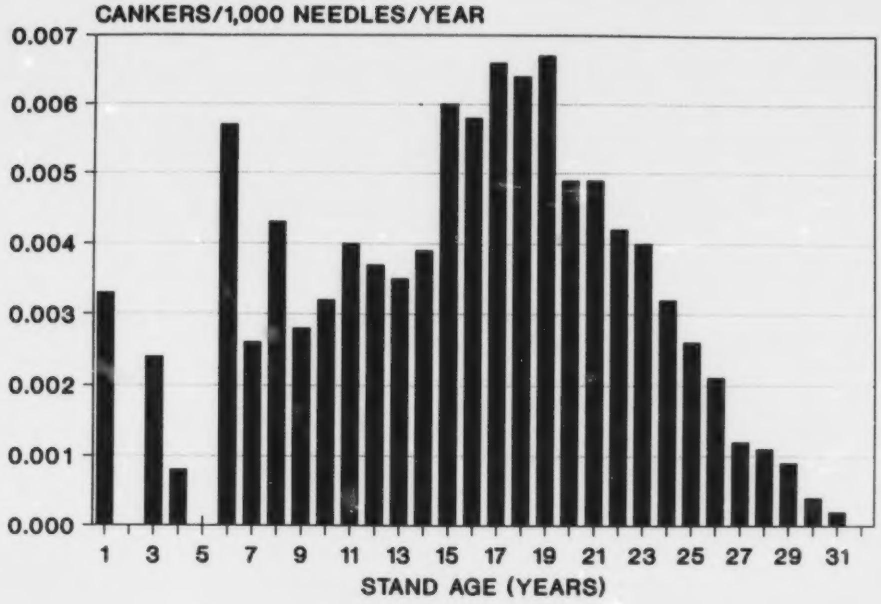

Figure 4. **Course of *C. ribicola* average annual rust index in an 84-tree sample of *P. monticola* from a natural stand located in Clearwater National Forest, Idaho.**

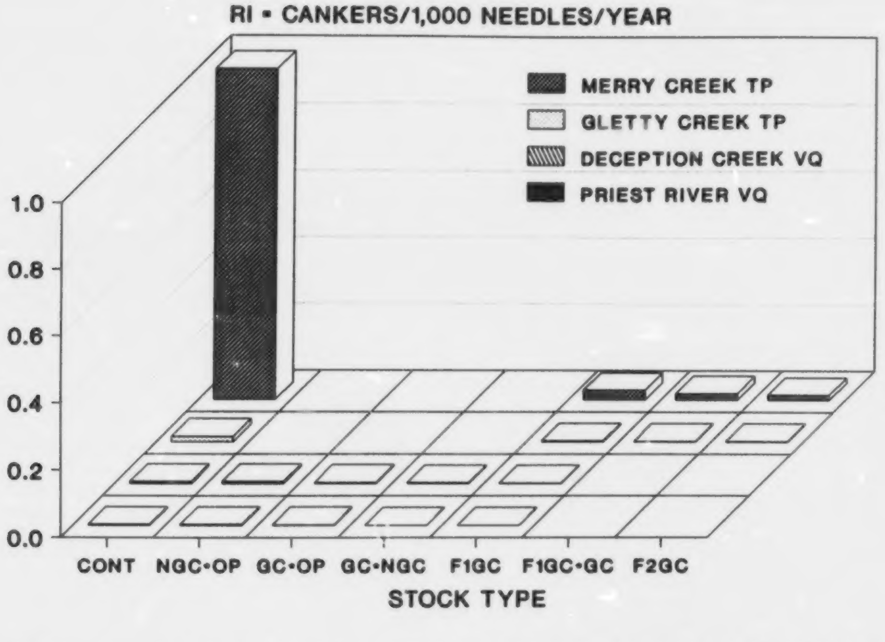

Figure 5. **Blister rust index for four *P. monticola* plantations growing in national forests in northern Idaho and eastern Washington.** Stock types ranging from susceptible to resistant. Blanks were not planted.

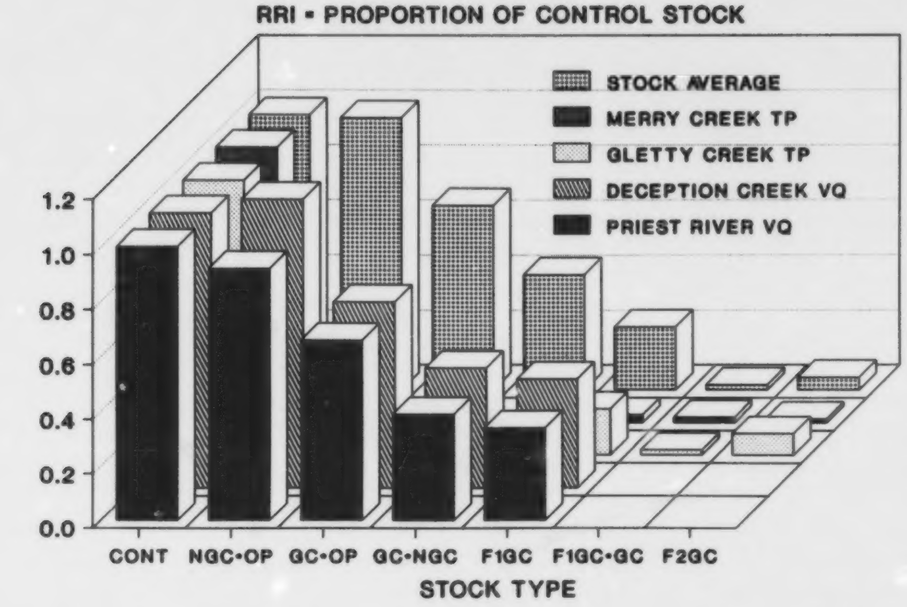

Figure 6. **Relative blister rust index (resistant and control) for four *P. monticola* plantations growing in national forests in northern Idaho and eastern Washington.** Stock types ranging from susceptible to resistant.

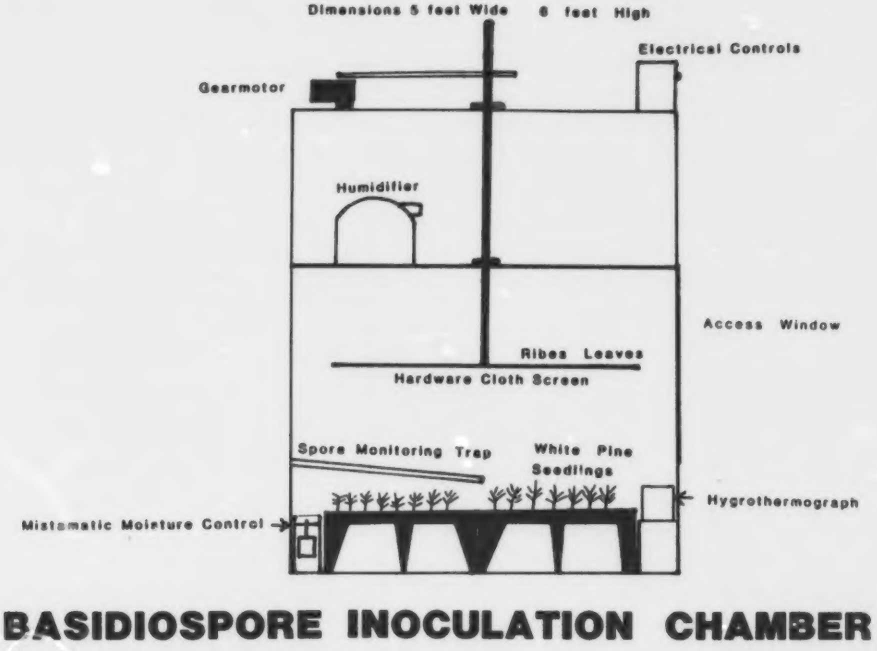
Dimensions 5 feet Wide 6 feet High
Electrical Controls
Gearmotor
Humidifier
Access Window
Ribes Leaves
Hardware Cloth Screen
Spore Monitoring Trap
White Pine Seedlings
Hygrothermograph
Mistomatic Moisture Control
BASIDIOSPORE INOCULATION CHAMBER

Figure 7. **The rotating-bed inoculation chamber is used to obtain quantitative blister inoculations of *P. lambertiana* and *P. monticola*.** The chamber is 150 cm wide and 180 cm high.

discovered that rotating the inoculum bed at 50 rpm reduced patterned deposition within the chamber and apparently resulted in a random distribution of basidiospores over the test bed.

The host materials for the inoculation reported in this paper were composed of nine sugar pine (*P. lambertiana* Dougl.) families and three western white pine families. Seeds were obtained from inventories maintained at Forest Service Pacific Northwest Region Dorena Tree Improvement Center. That facility is located in west-central Oregon near Cottage Grove, and has been described (McDonald et al. 1984). Seedlings were grown at Moscow, Idaho, in super pine cells inside a greenhouse heated to maintain a minimum winter temperature of 2°C.

Inoculum was obtained from the Dorena *Ribes* garden, which had been inoculated with a composite collection of aeciospores from the Oregon Cascades, including the Champion Mine area (McDonald et al. 1984). In mid-August 1983, the second growing season for the seedlings, the inoculation was conducted using two chambers. Ninety-six seedlings (eight per family × 12 families) were randomly placed in each of two pine cell racks (30 cm × 60 cm) to provide 16 seedlings per family in two replications within a 3600-cm^2 area for each run and chamber combination. The families were given eight spore concentrations in inoculation runs x inoculation chamber combinations over a range of 613 to 18 210 spores/cm^2. Spore concentration was controlled by monitoring the single trap shown in Figure 7. When repeated reading of fresh slides placed in the monitoring position summed to a predetermined density (low = ca 500, moderate = 2000 to 5000, and high = over 10 000 spores/cm^2), the two racks of seedlings were removed from the chamber, misted, wrapped in plastic film, and placed on the greenhouse bench for an additional 24 hours. The next batch of seedlings was then placed in the chamber and, if needed, new inoculum was added to the bed. The cycle was repeated continuously until four runs were completed.

Spore concentration actually deposited was estimated by seven rubber-cement-coated slides randomly placed over the 3600-cm^2 target area. These slides were removed upon seedling replacement and were stained in glycerine-aniline blue. Sixty fields per slide were counted under a compound microscope at 200 to 400× depending on spore density. The low target yielded monitor = 631 and random = 613 for one run. A second low run gave monitor = 920 and random = 800. The moderate concentration targets gave monitor-random combinations of 3413 versus 3224, 3141 versus 2342, 2191 versus 1919, and 5232 versus 4234. The high targets gave combinations of 10 192 versus 6670 and 10 192 versus 18 210. The monitor slide served its purpose of giving a wide spread of spore densities. It was also obvious that a single slide would not be sufficient to quantify spore cast in these chambers over the 3600-cm^2 target area.

ARTIFICIAL INOCULATION: RESULTS

Seedlings were inspected for needle spots monthly from 9 to 18 months after infection. Infection frequency in June, after inoculation the previous August, was computed as average spots per tree over the 16-seedling family computed from spot counts on total tree foliage (Fig. 8). Variation of infection frequency does not contain much useful information because host-controlled factors such as number of needles, needle length, and number of rows of stomata induce sources of variation. Other uncontrolled factors are host-rust genetic factors and rust environment factors such as spore development history and the environment at time of spore deposition, germination, and penetration. In addition to the control of spore concentration, if one could control all these sources of variation, then a stable measure of infection should result. Such a measure would remain constant over spore concentration and vary in predictable ways with all the factors discussed above.

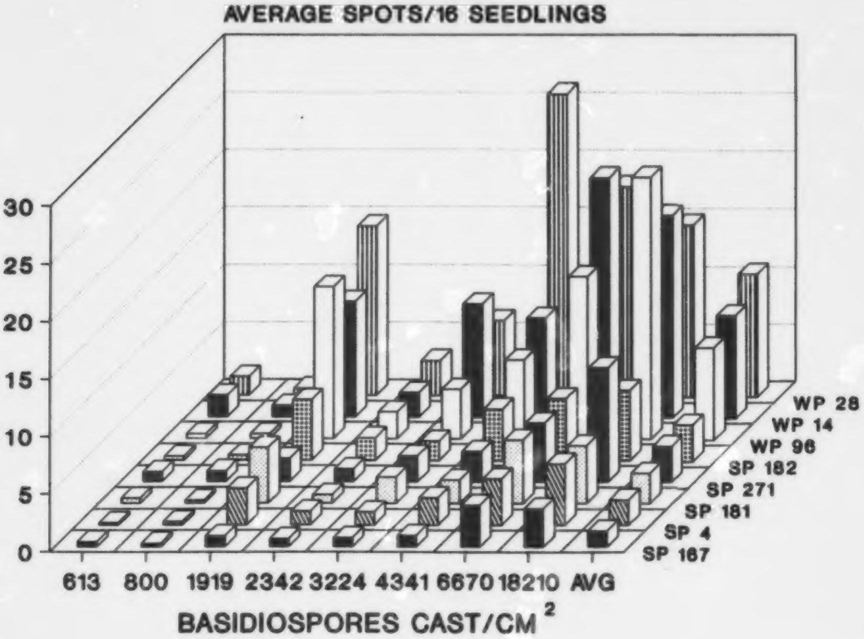

Figure 8. Frequency of *C. ribicola* needle lesions on five families of *P. lambertiana* (SP) and three of *P. monticola* (WP) 10 months after artificial inoculation at eight spore densities.

In this experiment, some host environment factors were controlled by counting number of needles exposed per tree, measuring needle length, and counting number of rows of stomata per needle. Host-rust genetics was controlled to some extent by using certain pine families and one population of rust inoculum. The largest source of variation was in the rust environmental factors of spore history and infection conditions, including the 24-hour period after spore delivery.

Needle size and stomata data were used to compute infective surface (McDonald et al. 1981) for each tree. Infection efficiency (spots per tree ÷ infective surface per tree ÷ average spore density for 3600-cm^2 target area) was then calculated for each tree and averaged over family and run-chamber combinations. The result was a considerable leveling of variation over spore concentration, allowing for the observation of some of the underlying sources of variation in the infection process (Fig. 9). The influence of spore concentration on infection was confounded by spore history and infection conditions, from chamber to chamber, and run to run (Fig. 9). The run-chamber combination that gave 1919 spores/cm^2 seems to stand out by yielding a higher infection efficiency over most families (Fig. 9). However, host species and family differences are clearly evident.

One way to compare families is by calculating relative infection efficiency (*RIE*) or the ratio of all remaining families to the most susceptible family (Fig. 10). About a 10× difference was observed between the lowest and highest *RIE* (Fig. 10) and this value agrees favorably with the 10× difference shown in a large population of western white pine families (Hoff and McDonald 1980). *RIE* could be calibrated with the field-estimated *RRI* so that the latter could be estimated in the laboratory. The calibration step would forge a direct connection between field performance and artificial inoculation, provided that the proper inoculation technique is used.

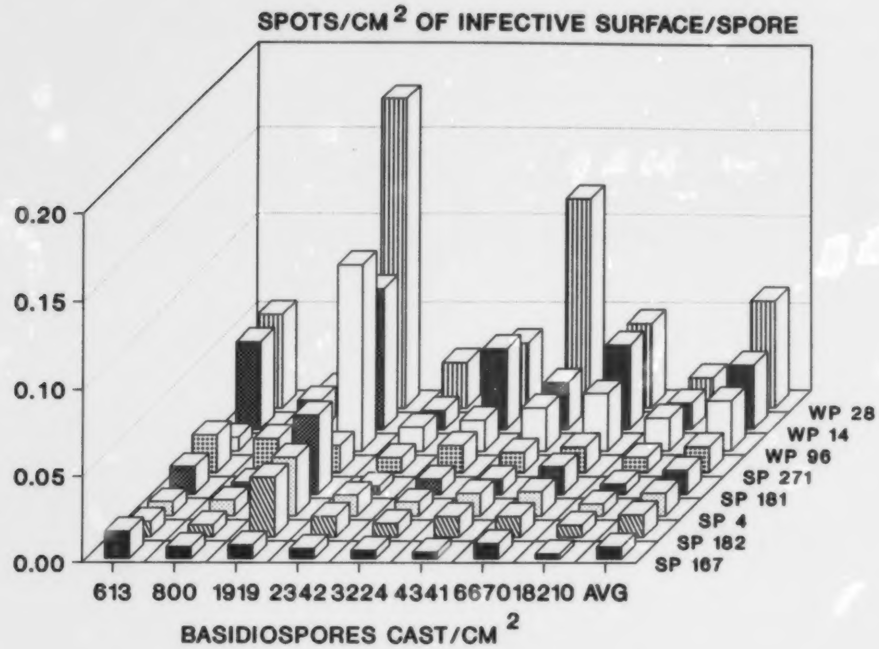

Figure 9. Infection efficiency of *C. ribicola* basidiospores on five families of *P. lambertiana* (SP) and three of *P. monticola* (WP) 10 months after artificial inoculation at eight spore densities.

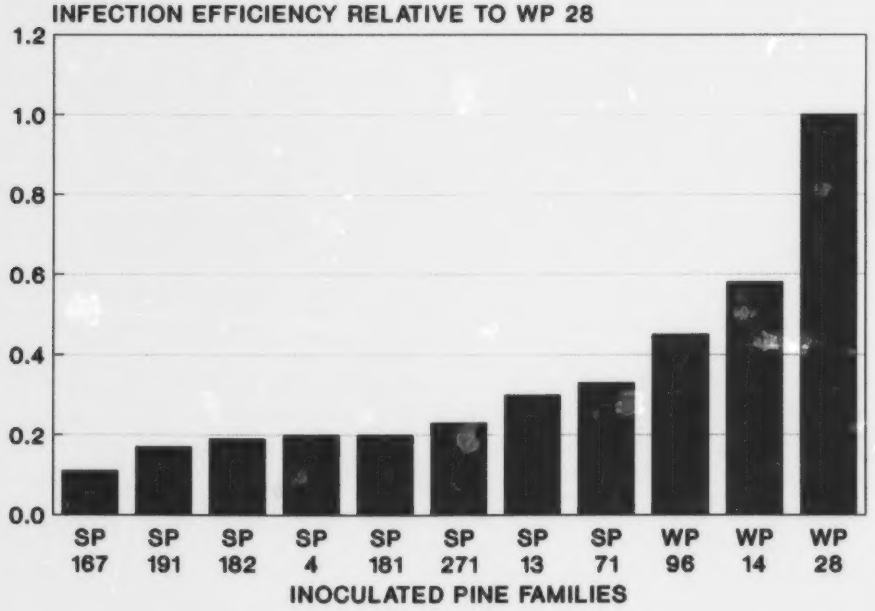

Figure 10. The *C. ribicola* infection efficiencies relative to the most susceptible *P. monticola* (WP) family observed on eight *P. lambertiana* (SP) and two *P. monticola* families (values averaged over all inoculum densities).

SPATIAL CONSIDERATIONS

The principal element still missing was a way to relate the components of the disease triangle to expected stand performance of given materials on a given site. In other words, if we could accurately predict the numbers of infections expected in a given situation of spore load, environment, and host-rust combination, how would we translate this knowledge into proportion of the stand infected or the probability of a tree becoming infected? Our first attempt was construction of a nonlinear regression of proportion of a stand infected on average number of infections/tree in the stand (McDonald et al. 1981). The similarity of this regression with the curve shape of the multiple-infection transformation (Fig. 11) (Van der Plank 1975) prompted a more thorough look at the epidemiological literature. The transformation is defined by Bald (1970, p. 38) "If y = the probability of finding one lesion or more on a root system, the proportion of infected root systems in a group of N susceptible plants growing in the same infected soil, will be given by an equation of the form

$$y = 1 - e^{-m} \qquad (3)$$

Here e is the base of natural logarithms and m is the number of lesions on the N plants, divided by N." For this relationship to hold, several assumptions must be made (Bald 1970; Grogan et al. 1980; Baker and Drury 1981). First, a single propagule must be capable of causing disease. Second, the host and pathogen are assumed to exhibit uniform susceptibility and virulence. Third, distribution of inoculum is random. Fourth, if microclimate is uniform, distribution of infection is also random. The transformation is commonly used to estimate the number of hits or infection points when the proportion of a population

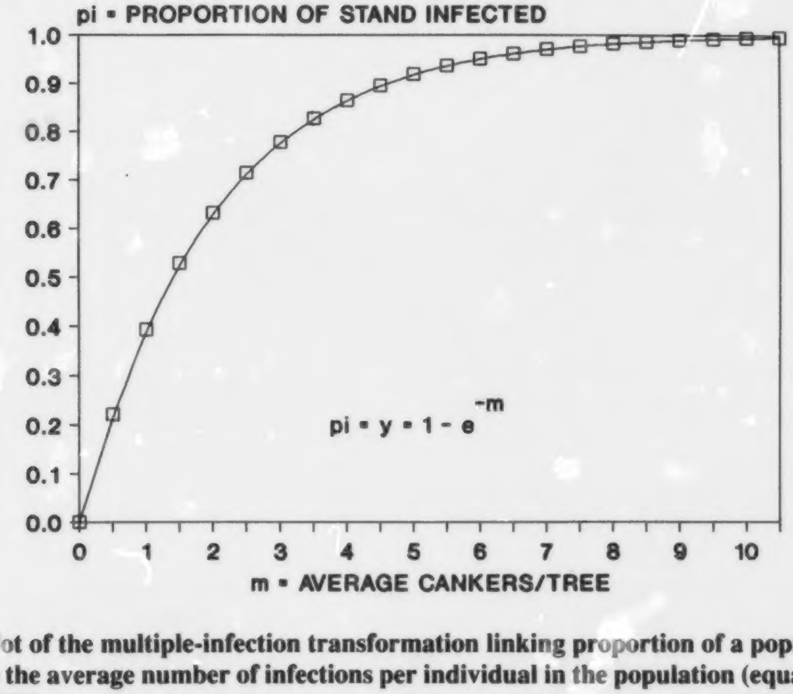

Figure 11. Plot of the multiple-infection transformation linking proportion of a population infected to the average number of infections per individual in the population (equation from Bald 1970).

infected is known (Van der Plank 1975). The relationship meets the expected curve when the assumptions are not violated. If any of the assumptions are violated, the distribution deviates from its theoretical form (Bald 1970).

In white pine stands growing in heterogeneous environments, spores are not distributed at random and the environment is not uniform. A deviation factor to quantify the actual deviation of a given population of white pine from the expected distribution was suggested by Fracker (1936). He empirically derived an equation to compute a deviation factor (*d*) which had the form

$$d = (m - p)/(p \times p) \tag{4}$$

where
m = actual average number of cankers per tree, and
p = expected number of cankers per tree from multiple infection equation [$p = -\ln(1 - y)$].

Fracker (1936) describes in detail how a clumped distribution of *Ribes* bushes would cause a deviation and how widely distributed bushes would lead to a very small deviation. These results gave us the idea of rewriting the multiple-infection transformation and then solving for the deviation factor so that, in essence, Fracker's empirical equation would have a connection to theory. The form follows

$$pi = 1 - e^{-[m/(1 + d \times m)]} \tag{5}$$

where
pi = y = proportion of stand infected
m = average number of cankers per tree, and
d = parameter to quantify deviation from theory.

If one solves equation 5 for *d*, then

$$d = \frac{m - [-\ln(1 - pi)]}{m \times [-\ln(1 - pi)]} \tag{6}$$

where
m = actual average number of infections per tree
$[-\ln(1 - pi)]$ = expected number of infections per tree

and
d = 0 when infections randomly distributed
d < 0 when infections tend to uniform distribution, and
d > 0 when infections tend to aggregated distribution.

The Northern Region Forest Service data mentioned earlier (Carlson and Toko, unpublished) and equation 6 were used to estimate the average deviation in northern Idaho white pine stands (Fig. 12). Most of this deviation is likely due to the variation in infection microclimate, distribution of basidiospore load, or both. Because microclimate tends to be quite uniform over large areas during the pine infection periods in northern Idaho, because *Ribes* bushes tend to be clumped in their geographic distribution, and

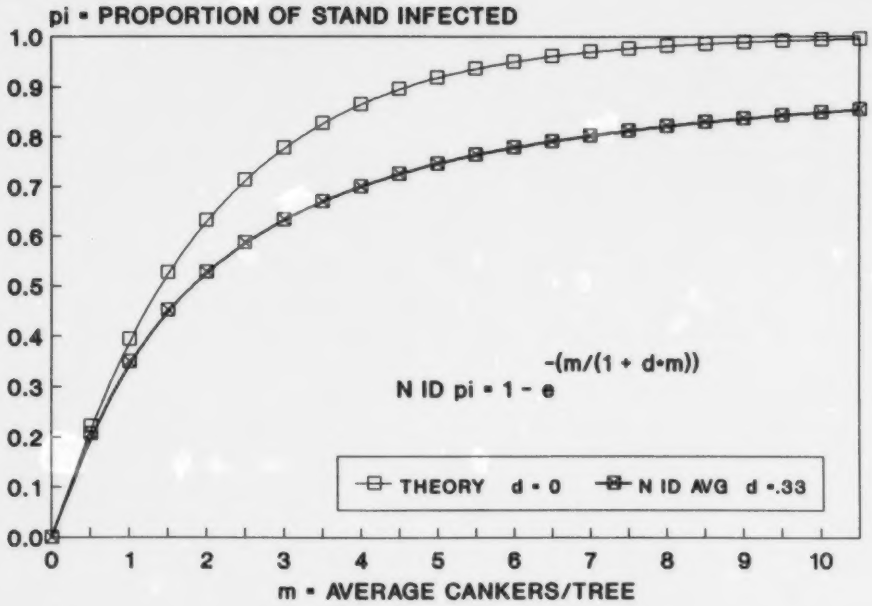

Figure 12. Comparison of theoretical and northern Idaho average forms of multiple-infection transformation. See text for modified equation.

because microclimate controlling the buildup of urediospores tends also to be geographically clumped (McDonald et al. 1981), the most likely influencing factor is variation in spore load. Basidiospores are assumed to meet the single-propagule requirement.

The factor exercising a major influence on the multiple-infection transformation that we have not yet discussed is distribution of immunity genes (genes controlling resistance mechanisms that prevent the establishment of macrosymptoms). The breeding program conducted in northern Idaho produced populations composed of several combinations of resistance mechanisms (McDonald and Hoff, these proceedings). Mechanisms such as fungicidal short shoot (Hoff and McDonald 1971) and premature needle shed (McDonald and Hoff 1970), which are the most common mechanisms in the resistance population to date (Hoff et al. 1973), prevent the formation of macrosymptoms (cankers). Thus, one ought to be able to quantify the epidemiologic function of these mechanisms by estimating the portion of *d* that is due to resistance.

The deviation factor was determined for the same test plantations used for the estimation of rust index discussed earlier. The raw results show the expected trend in *d* with increasing resistance (Fig. 13). The site-related influences on distribution of spore load and microclimate were removed by computation of relative deviation factor (*rd* = *d* for resistant stock/*d* for controls) (Fig. 14). The stock type performed as expected with increasing levels of resistance showing higher deviations. When computed values for *rd* were incorporated into equation 5, the resulting family of curves depicts the transformation expected for stock of varying levels of immunity resistance (Fig. 15).

Values of *d* were also computed for the western white pine and sugar pine families inoculated in the rotating bed chambers. Values of *rd* encompass the same spread as did those determined in the

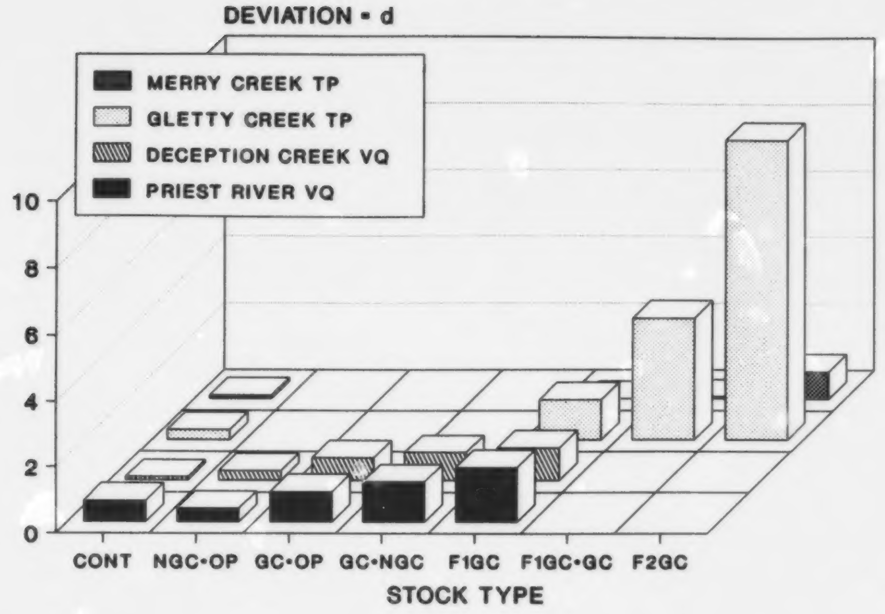

Figure 13. Multiple-infection transformation deviation factors observed for four white pine plantations growing in national forests in northern Idaho and eastern Washington. Stock types ranging from susceptible to resistant.

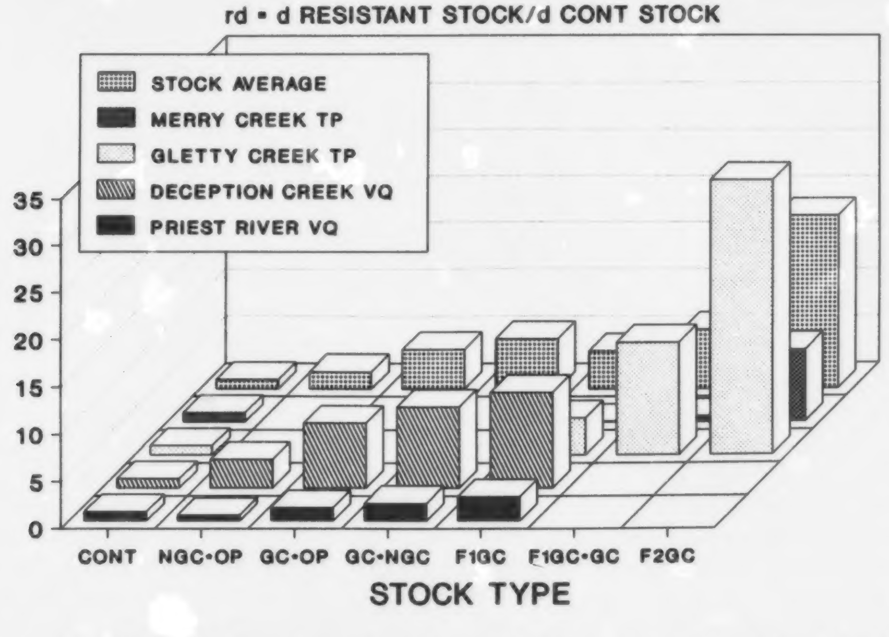

Figure 14. Multiple-infection transformation relative deviation factors (deviation of resistant stock and deviation of control stock) observed for four *P. monticola* plantations growing in national forests in northern Idaho and eastern Washington. Stock types ranging from susceptible to resistant.

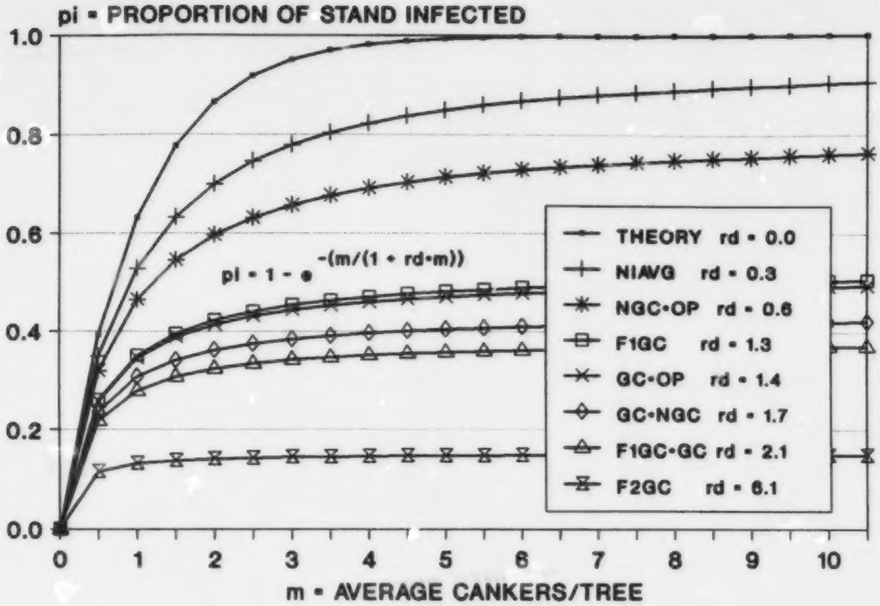

Figure 15. Comparison of theoretical, northern Idaho average, and six resistant-stock forms of multiple-infection transformation. See text for modified equations.

field test plantations (Fig. 16). Thus, one could expect that artificial inoculation of seedlings, under conditions that satisfy the multiple-infection transformation assumptions, will supply the stock performance parameters of *RI* and *d*.

RUST PROGRESS CURVES

The epidemiologic parameters of *RRI* and *rd* can then be used as predictors of stock performance, to test management scenarios, or remotely monitor disease development. A fundamental component of such models is the disease progress curve. The modified multiple-infection transformation was modified further and used to generate curves of rust progress by plotting percent infected (*pi*) against time rather than number of cankers. The equation takes the form

$$pi_t = 1 - e^{-[m_t/(1 + d \times m_t)]} \qquad (7)$$

where

pi_t = proportion of stand infected at age t, and

m_t = number of cankers accumulated by age t

= $ST_t \times RRI$ (see equation 2).

These curves are useful in predicting site-specific performance of various stock types in combination with other management options, such as control of stand density or *Ribes* population management (Hagle et al. 1989). Several curves illustrate the variety of stock type and site conditions

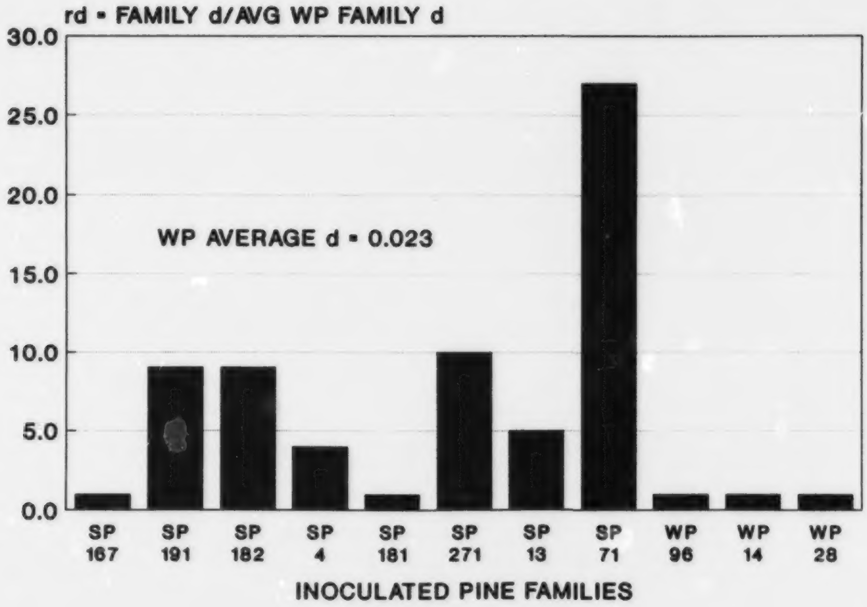

Figure 16. The *C. ribicola* multiple-infection deviations relative to the average for *P. monticola* (WP) families observed for eight *P. lambertiana* (SP) families (*d* values averaged over all inoculum densities).

found in northern Idaho white pine forests. If a forester wanted to decide if a silvicultural management scenario were feasible, he could ask a question about the outcome of variations in rust hazard in susceptible plantations. If we assume that *RI* takes the values 0.5, 0.015, and 0.0005 and that *d* values associated with these values are 0.1, 0.3, and 0.8 to represent high, average, and low hazard in northern Idaho, then we see that reducing *Ribes* density and clumping distribution to give $RI = 0.0005$ and $d = 0.8$ will afford an opportunity to manage susceptible white pine populations providing silvicultural options are applied (Fig. 17).

A forester could have an average hazard site and need to select a stock type or determine if an available stock was suitable. Suppose the forester wants to compare three stock-types, susceptible (wild), first-generation general combiners (F1GC), and the second-generation derived from general combiners (F2GC). The *RI* and *d* parameters associated with the improved stock are divided by the values of these parameters associated with the susceptible population at the site in question to generate *RRI* and *rd*. The curves show (Fig. 18) F1GC stock would be marginal. But, F1GC is preferable because it will moderate selection pressure to the rust population. Consequently, in terms of managing resistance genes, the best choice would be F1GC stock plus application of some silvicultural techniques.

Suppose a forester wanted to establish a plantation on a known high-hazard site. The question here is performance of the best material currently available--F2GC. The progress curve (Fig. 19) shows a very rapid increase in infection to about 40% by age 10. The forester might consider a species other than white pine, could reduce the *Ribes* population density, could increase planting density, or might investigate pruning of cankers from the F2GC stock. Finally, our forester wants to plant on a known low-hazard site and has available open-pollinated seed from phenotypically resistant trees known to be general

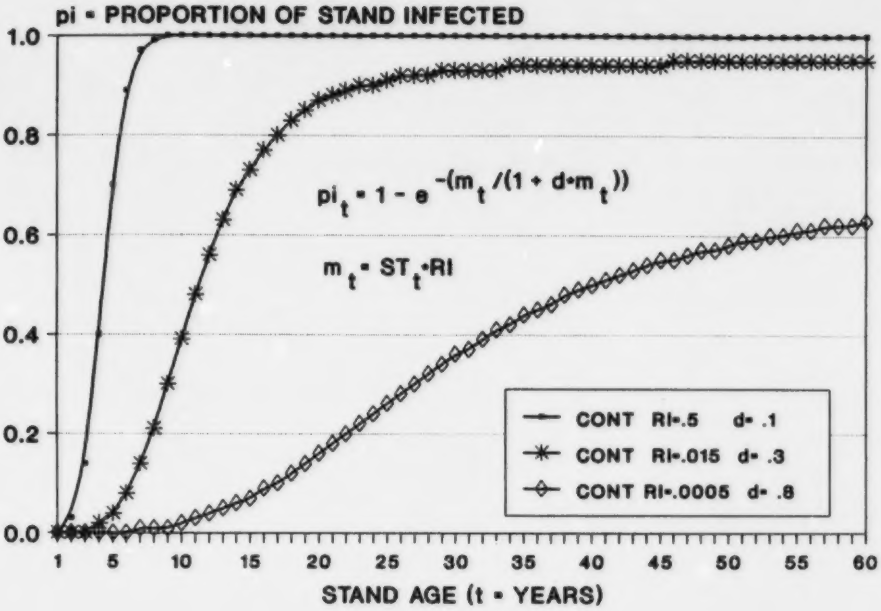

Figure 17. Performance of a wild susceptible (cont) population of *P. monticola* (WP) at high, average, and low blister rust hazards for northern Idaho and eastern Washington as predicted by the disease progress equation discussed in the text.

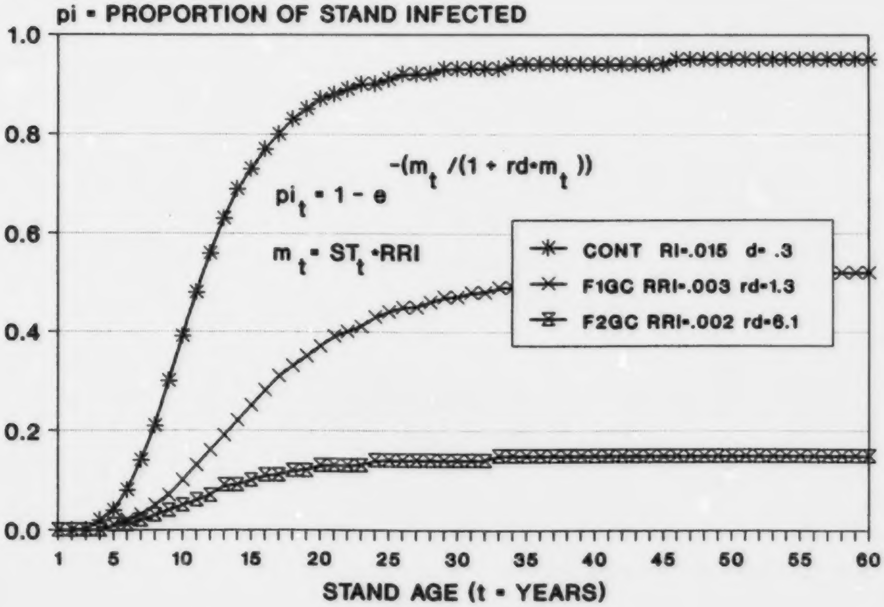

Figure 18. Performance of a wild susceptible (cont) population, a moderately resistant (F1GC) population, and a highly resistant (F2GC) population of *P. monticola* (WP) at an average blister rust hazard for northern Idaho and eastern Washington, as predicted by the disease progress equation discussed in the text.

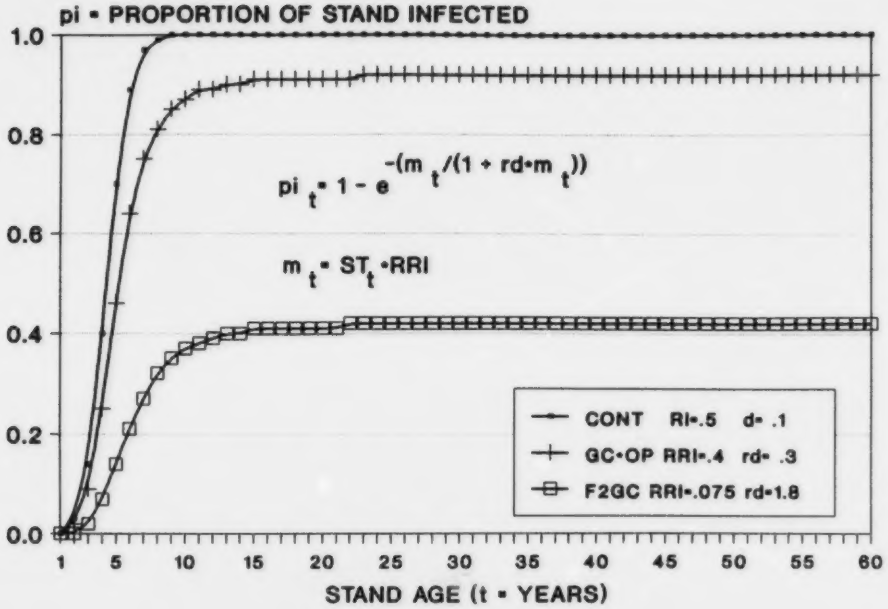

Figure 19. Performance of a wild susceptible (cont) population, a moderately resistant (GC × OP) population, and a highly resistant (F2GC) population of *P. monticola* (WP) at high blister rust hazard for northern Idaho and eastern Washington, as predicted by the disease progress equation discussed in the text.

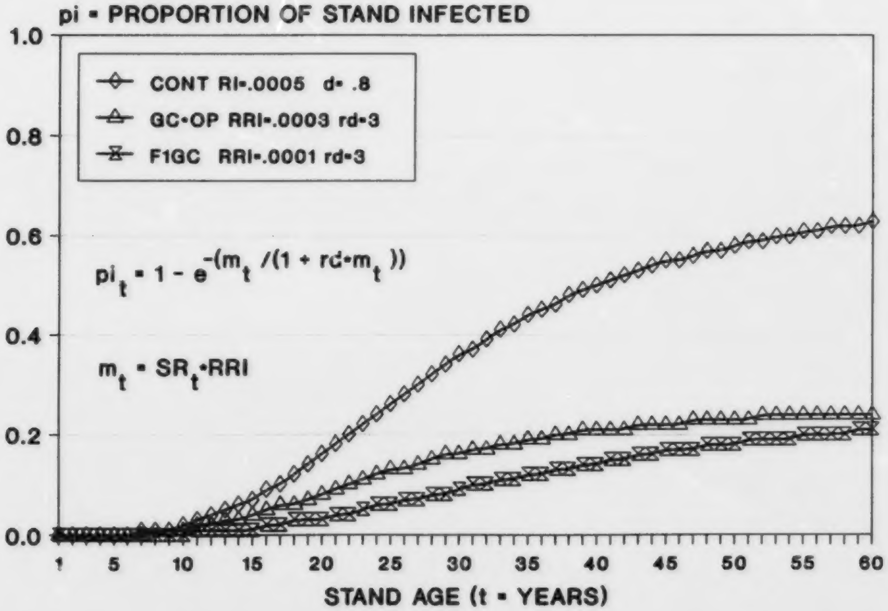

Figure 20. Performance of a wild susceptible (cont) population, a moderately resistant with broad genetic base (GC × OP) population, and a moderately resistant with narrow genetic base (F1GC) population of *P. monticola* (WP) at low blister rust hazard for northern Idaho and eastern Washington, as predicted by the disease progress equation discussed in the text.

combiners (GC*OP) and seed from a clonal orchard composed of these same selections (F1GC). Since the curves illustrate little difference between the resistant stocks (Fig. 20), the forester should select the population with the largest genetic breadth (GC*OP).

OTHER APPLICATIONS

Maximum utilization of this approach requires that hourly microclimate data be available. Either of two approaches or a combination are possible. First, a recording station or stations could be placed in the target stands and the actual weather record would be used to drive the models. Second, a correlative weather model, such as MTCLIM, could provide the input data.

Most promising of all is consolidation of weather, *Ribes*, rust, and pine models along with topographic and vegetation data into a dynamic geographic information system that would provide maps of expected hazard or disease development, much as forest evapotranspiration and photosynthesis have been mapped (Running et al. 1989). One should not overlook the possibility of using these ideas to create a generic model for the stem rusts of pine. Other possible applications of the ideas are critical selection of pines phenotypically resistant to pine rusts to reduce the possibility of choosing escapes, monitoring plantations to provide early warning of unacceptable infection levels, analysis of artificial inoculations in progeny testing, and assessment of integrated management strategies.

A word of warning is in order. The ideas discussed in this paper have high potential for application to all pine rusts; but, at this point, they are largely theoretical and should be subjected to a thorough program of verification before they are widely applied to either pine rust, generally, or white pine blister rust, specifically.

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POLYACRYLAMIDE ELECTROPHORESIS AND BIOCHEMICAL MARKERS IN LOBLOLLY PINE FOR RESISTANCE TO FUSIFORM RUST

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ABSTRACT

Pollen from various loblolly pine (*Pinus taeda* L.) families has been analyzed by polyacrylamide gel electrophoresis. A distinct isozyme pattern of glutamate-oxaloacetate transaminase (GOT) has been observed for each pollen sample collected from trees exhibiting high, intermediate and low resistance to fusiform rust, *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*. A similar isozyme pattern of GOT was also evident for megagametophytes of seeds collected from the same trees from which the pollen was obtained. No such correlation was observed for the embryos of the respective seeds. Cross-pollination experiments are carried out to prove conclusively the linkage between the particular isozyme pattern of GOT and resistance or susceptibility of a tree to fusiform rust from which the samples are collected.

INTRODUCTION

Pest resistance is one of the most important factors for the successful establishment of industrial plantations of trees. If trees have a high potential for fast growth and yield but low resistance to pests, maximum yields can not be obtained. Effective identification of desirable traits is crucial for a tree breeding program, however, inoculation tests to evaluate disease resistance are relatively time-consuming and costly. Identification of biological markers, directly or indirectly linked to important traits such as disease resistance or pathogen virulence, would be a valuable tool for the rapid screening of disease resistant plant material.

Several authors report correlations between specific isozymes and their patterns that are linked directly or indirectly to disease resistance (Rick and Frobes 1974; Sheen and Diachun 1978; Weeden and Marx 1984). Bernier et al. (1983) and Alfenas et al. (1983) were able to differentiate by polyacrylamide electrophoresis virulent and avirulent strains of *Ophiostoma ulmi* (Buism.) Nannf. and *Cryphonectria cubensis* (Bruner) Hodges., respectively. This technique proved also very useful in the separation of biological species of *Armillaria* spp. (Lin et al. 1989) as well as the inter- and intraspecific differentiation within the genus *Cronartium* (Powers et al. 1989).

The present paper reports on work in which polyacrylamide gel electrophoresis has been used as the essential tool for the identification of biological markers linked to specific traits such as resistance in loblolly pine to fusiform rust. It provides also some additional information on the characterization of families of the loblolly pine as well as several ramets within clones.

MATERIALS AND METHODS

Pollens

Pollen samples from loblolly pine selections resistant or susceptible to *Cronartium quercuum* f. sp. *fusiforme* (Table 1) were used in isozyme analysis. Pollen samples were kept in sealed ampoules at 4°C until used. The procedures for isozyme analysis were the same as described previously (Powers et al. 1989). Pollen samples from ramets of clones 42R, 4625-3, and 4666 were also included in these studies (Table 2).

Seeds

The seeds were surface sterilized with 70% ethyl alcohol and stratified at 4°C fcr 8 weeks. Following stratification, an average of 50 seeds from each family (Table 3) were placed in petri dishes on filter paper moistened with distilled water. The seeds were left to germinate in an incubator set for 16 hours of light and 8 hours of dark (McLemore and Czabator 1961). Germination occurred after 10 days of incubation. When seed coats were opened, the germinated seeds were collected. Embryos were removed from the megagametophytes and kept frozen in a deep freeze until all seeds were collected. The procedures for isozymes analysis were the same as those for pollen.

RESULTS

Pollen

Out of 23 isozymes tested, 13 show positive reactions to the pollen samples. Some of the enzymes show differences among the families, others do not differ from family to family (Table 4). Pollen of loblolly pine families resistant, intermediate, and susceptible to *Cronartium quercuum* f. sp. *fusiforme* can be distinguished by their glutamate-oxaloacetate transaminase (GOT) patterns (Fig. 1). According to the GOT patterns, the group of the resistant family is separated from the other groups by the presence of GOT bands at a Rf of 0.32. Groups of intermediate and susceptible families are separated by a GOT band at Rf 0.22. All pollen samples from loblolly pine have GOT bands at Rf 0.25 and 0.28. Figure 2 shows the regrouping of these loblolly pine families based on their GOT isozyme patterns. Their corresponding disease ratios (D/R) are given in Table 5.

After sorting families, based on the GOT isozyme pattern of the pollen samples, into resistant, intermediate and susceptible groups, the families can be further characterized by their isozyme pattern of glycerate-2-dehydrogenase (G_2DH) and 6-phosphogluconate dehydrogenase (6PGD). For example, within the susceptible group, family 3470-16 is shown by its 6PGD bands at Rf 0.14 and 0.16, whereas family 152-254 is lacking the 6PGD band at Rf 0.07 (Fig. 3). For the rest of the group, family 4666-4 has its G_2DH band at Rf 0.22, whereas families 4624-4 and 4625-3 have a band at Rf 0.27 (Fig. 4).

Table 1. **Disease ratio (D/R) of loblolly pine families used in isozyme studies of pollen**

No.	Family	D/R
1	4666-4	1.04
2	7840-16	0.61
3	42R	0.55
4	3470-16	1.11
5	152-201	0.44
6	152-435	0.54
7	152-196	0.38
8	152-254	0.86
9	4624-4	1.05
10	152-407	0.24
11	151-740	0.65
12	7896-22	0.60
13	152-339	0.44
14	4625-3	0.66
15	300-33-7	0.58

Table 2. **List of ramets of clones used in isozyme studies**

No.	Clone	Ramet
1	4625-3	No. 4
2	42-R	No. 1
3	4666-4	No. 3
4	4625-3	No. 3
5	42-R	No. 3
6	4625-3	No. 2
7	4625-3	No. 1
8	4666-4	No. 2
9	4625-3	No. 5
0	42-R	No. 2
1	42-R	No. 5
2	42-R	No. 4
3	4666-4	No. 1

Table 3. **Loblolly pine families used in isozyme studies of pollen, embryos and female gametophytes**

No.	Family
1	151-26
2	152-196
3	151-183
4	151-112
5	301-29-5

Table 4. **List of enzymes tested on loblolly pine pollen and seeds**

Enzyme	Reaction[a]	Difference[b]
A. Hydrolases, lyases and transferases		
Acid phosphatase (AP)	(–)	
Aldolase (Ald)	(–)	
Alkaline phosphatase (AkP)	(–)	
Esterases (EST)	(+)	(+)
Leucine aminopeptidase (LAP)	(+)	(–)
Phosphoglucomutase (PGM)	(+)	(+)
B. Dehydrogenases		
Alcohol dehydrogenase (ADH)	(–)	
Glucose-6-phosphate dehydrogenase (G_6PDH)	(+)	(–)
Glycerate-2-dehydrogenase (G_2DH)	(+)	(+)
Glycerophosphate dehydrogenase (GPDH)	(–)	
Isocitrate dehydrogenase (IDH)	(–)	
Lactate dehydrogenase (LDH)	(+)	(–)
Malate dehydrogenase (MDH)	(+)	(–)
6-Phosphogluconate dehydrogenase (6PGD)	(+)	(+)
Sorbitol dehydrogenase (SDH)	(–)	
C. Others		
Adenylate kinase (AK)	(–)	
Fumarase	(–)	
Glucose phosphate isomerase (GPI)	(+)	(–)
Glutamate oxaloacetate transaminase (GOT)	(+)	(+)
Hexokinase (HEX)	(+)	(+)
Malic enzyme (ME)	(+)	(–)
Peptidase (PEP)	(+)	(–)
Peroxidase (PO)	(+)	(+)

[a] + = positive reaction; - = negative reaction.

[b] + = difference; - = no difference.

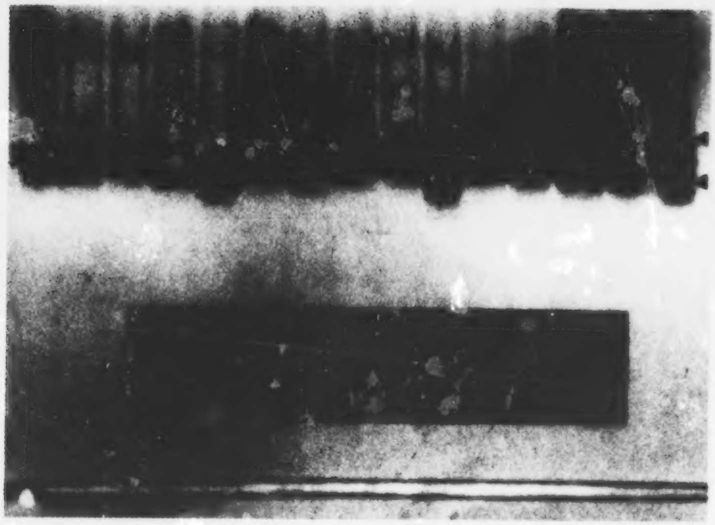

Figure 1. Isozyme pattern of glutamate-oxaloacetate-transaminase.

1. 4666-4
2. 7840-16
3. 42R
4. 3470-16
5. 152-201
6. 152-435
7. 152-196
8. 152-254
9. 4624-4
10. 152-407
11. 151-740
12. 7896-22
13. 152-339
14. 4625-3
15. 300-33-7

△ Rf: 0.25, 0.28
▲ Rf: 0.22, 0.32

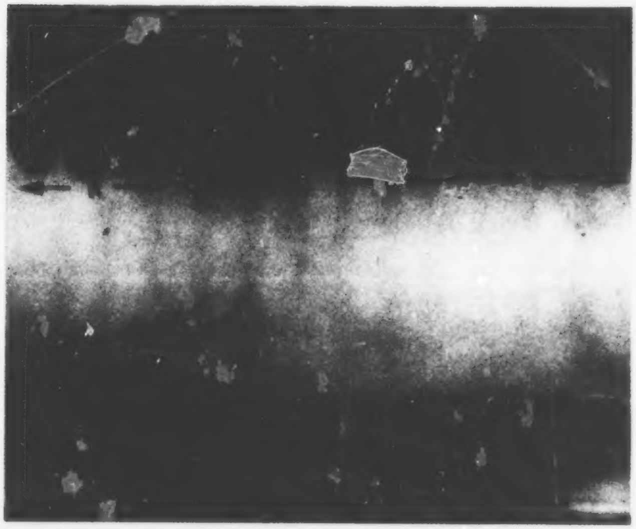

Figure 2. Isozyme pattern of glutamate-oxaloacetate-transaminase.

Resistant	*Intermediate*	*Susceptible*
1. 152-196	4. 152-339	10. 4625-3
2. 152-201	5. 152-435	11. 152-254
3. 300-33-7	6. 42R	12. 4624-4
	7. 7896-22	13. 4666-4
	8. 7840-16	
	9. 151-740	

Table 5. **Correspondence of GOT pattern and disease ratio (D/R) of loblolly pine families**

No.	Family	D/R	GOT pattern
1	152-407	0.24	Resistant
2	152-196	0.38	Resistant
3	152-201	0.44	Resistant
4	300-33-7	0.58	Resistant
5	152-339	0.44	Intermediate
6	152-435	0.54	Intermediate
7	42R	0.55	Intermediate
8	7896-22	0.60	Intermediate
9	7840-16	0.61	Intermediate
10	151-740	0.65	Intermediate
11	4625-3	0.66	Susceptible
12	152-254	0.86	Susceptible
13	4624-4	1.05	Susceptible
14	4666-4	1.04	Susceptible
15	3470-16	1.11	Susceptible

Figure 3. Isozyme pattern of 6-phosphogluconate dehydrogenase.

1. 152-201
2. 152-407
3. 300-33-7
4. 7840-165
5. 42R
6. 152-435
7. 152-196
8. 151-740
9. 7896-22
10. 152-339
11. 4666-4
12. 3470-16
13. 152-254
14. 4624-4
15. 4625-3

▲ Rf: 0.07, 0.14, 0.16

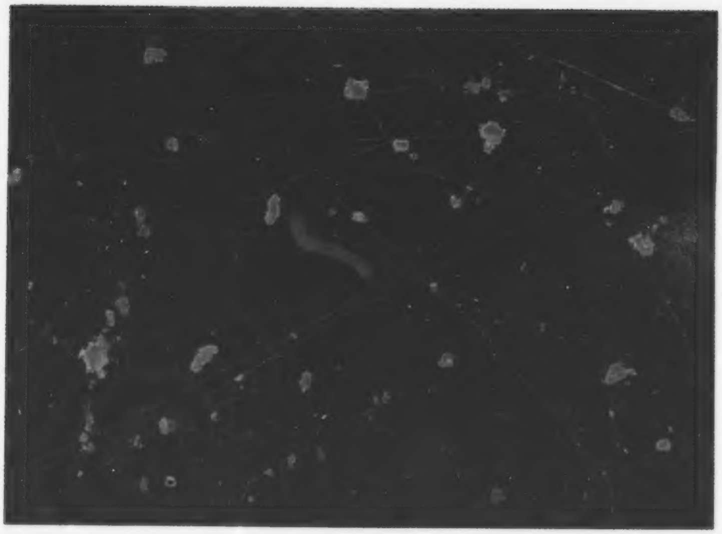

Figure 4. Isozyme pattern of glycerate-2-dehydrogenase.

1. 152-201
2. 152-407
3. 300-33-7
4. 7840-165
5. 42R
6. 152-435
7. 152-196
8. 151-740
9. 7896-22
10. 152-339
11. 4666-4
12. 3470-16
13. 152-254
14. 4624-4
15. 4625-3

▴ Rf: 0.22, 0.27

In addition, pollen samples collected from ramets of clones 42R, 4625-3, and 4666-4 can also be identified by their GOT isozyme patterns. Ramets of clone 42R differ from the others by the presence of a band at Rf 0.22, whereas ramets of clone 4666-4 have an additional band at Rf 0.18 comparable to clone 4624-4 (Fig. 5).

Embryos, Megagametophytes and Pollens

Further comparisons between isozyme patterns of pollens, embryos and megagametophytes indicated that the isozyme patterns of GOT, G_2DH, and PGM of megagametophytes are similar to that of pollen (Fig. 6). The isozyme patterns of embryos are different from those of pollen and megagametophytes in respect to the pattern that indicates disease resistance, as well as that of characteristic family traits. Furthermore, the isozyme pattern of peroxidase shows much lower activity from pollen samples than that of embryos and megagametophytes.

DISCUSSION

A linkage between isozyme patterns of GOT from pollen families of loblolly pine and their resistance to fusiform rust has been observed. Pollen samples from loblolly pine families resistant and susceptible to *C. quercuum.* f. sp. *fusiforme* displayed specific isozyme patterns of GOT that were linked to susceptibility or resistance of the tree from which the pollen was collected (Powers et al., 1986). Since pollens carry only half of the genetic information of the tree, it seemed necessary to extend these studies to the isozyme patterns of the female gametophytes and embryos in order to improve our picture of the

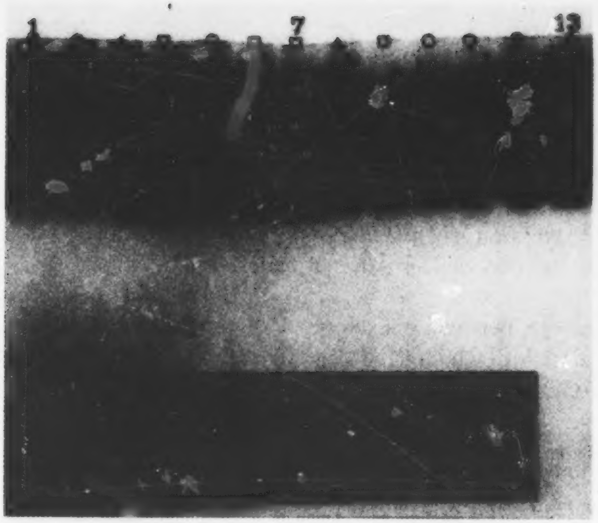

Figure 5. Isozyme pattern of glutamate-oxaloacetate-transaminase.

○ Ramets of clone 42-R : 2,5,10,11,12
□ Ramets of clone 4625-3: 1,4,6,7,9
△ Ramets of clone 4666-4: 3,8,13

▲ Rf: 0.18, 0.22

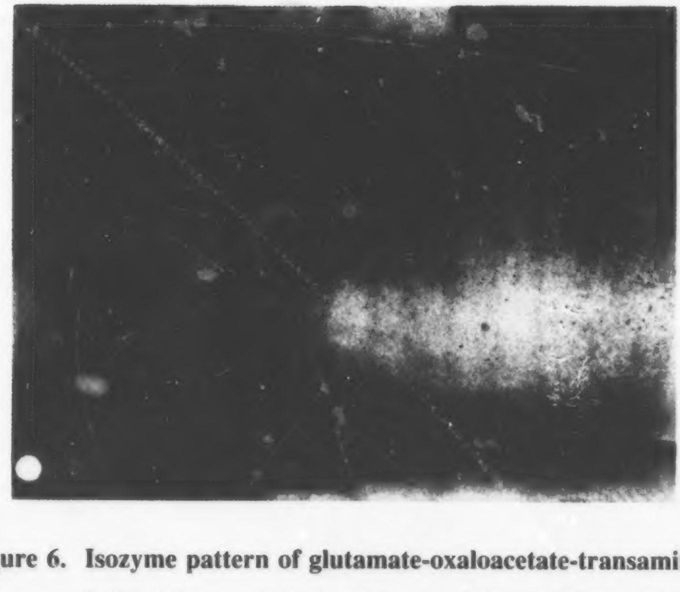

Figure 6. Isozyme pattern of glutamate-oxaloacetate-transaminase.

Pollen: 1-5 Embryos: 6-10 Megagametophytes: 11-15 resistance mechanisms involved. Results from these studies indicate that both pollen and megagametophytes show similar GOT isozyme patterns. Therefore, we assume that it is possible to use isozyme pattern of pollen samples for screening of resistant individuals within families of loblolly pine.

The lack of clear differences in GOT isozyme pattern of embryos between resistant and susceptible families may be partially explained by the fact that the seeds used in this study came from open-pollinated trees. Further work will be carried out to study the isozyme patterns of control pollinated seed from each family. In addition, such studies may provide information as to whether inheritance of resistance is influenced by the maternal or paternal parent.

Cross-pollinations between resistant and susceptible families of loblolly pine were carried out in 1988 to obtain material with which to finally establish whether the genetic inheritance of resistance is indeed linked with the GOT isozyme pattern. Once such a linkage between resistance and the GOT isozyme pattern is conclusively established, it should provide a simple and rapid bioassay for the selection of rust resistant loblolly pine.

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GALL SHAPE AS AN INDICATOR OF RESISTANCE TO FUSIFORM RUST IN LOBLOLLY PINE

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ABSTRACT

Fusiform rust galls were examined in loblolly pine plantations in Texas, Louisiana, Mississippi, Florida, and Georgia to determine if gall shape differed by geographic seed source and planting location. Gall length and width were measured and a gall form value (gall length/gall width) was derived for each gall. Trees grown from Texas and Livingston Parish, Louisiana, seed sources tended to have low gall form values (round galls) at some locations but not at others. In Early and Greene counties, Georgia, the Livingston Parish trees are resistant to fusiform rust and gall form values were low for this seed source. In contrast, higher gall form values were obtained for three separate plantings of Livingston Parish trees in Madison County, Florida, where this stock is very susceptible to *Cronartium quercuum* f. sp. *fusiforme*. Gall form values were generally high for all plantings of Mississippi, Georgia, and Florida seed sources. Gall shape may be a useful parameter in evaluating the response of field-grown pines against fusiform rust.

INTRODUCTION

The shape of galls caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* is highly variable on field-grown loblolly (*Pinus taeda* L.) pines. Some galls are elongated, many are very irregularly shaped, and others are round. The elongated and irregularly shaped galls cause the most damage because they affect more of the tree than round galls do. Long galls that originate in branches often grow into the stem; round branch galls are usually less damaging because they are restricted to the branch.

Gall shape is a useful parameter for evaluating seedlings that have been inoculated in tests for rust resistance because round gall formation appears to be an expression of resistance in pine seedlings (Snow et al. 1980). If the same is true for field-grown trees, one would expect a tendency for galls to be round in the populations of loblolly pine from Livingston Parish, Louisiana, and all natural forests west of the Mississippi River that were identified as resistant to fusiform rust by Wells and Wakeley (1966) and Grigsby (1973).

The purpose of this paper is to report the results of a survey made for gall shape in geographically resistant and susceptible loblolly pine populations.

METHODS

Twenty-five loblolly pine plantations, located from Texas to Georgia, were surveyed for gall shape (Table 1). The seed sources of the trees in all the plantations were known: 14 of the plantings were

Table 1. Average gall form values for fusiform rust galls in 25 loblolly pine plantings[a]

Planting location	Seed source	Number of galls	Mean gall form
Harrison Co., MS	Mississippi	50	3.78
Greene Co., GA	Georgia	49	4.04
Marion Co., FL	Florida	49	3.76
Jackson Co., FL	Florida	49	3.81
Marion Co., FL	Florida	50	3.27
Livingston Parish, LA	Livingston Parish, LA	35	3.29
Pearl River Co., MS	Livingston Parish, LA	100	3.20
Harrison Co., MS	Livingston Parish, LA	50	3.50
Jackson Co., FL	Livingston Parish, LA	50	3.85
Early Co., GA	Livingston Parish, LA	52	2.65
Jefferson Co., GA	Livingston Parish, LA	50	3.08
Greene Co., GA	Livingston Parish, LA	32	2.93
Wheeler Co., GA	Livingston Parish, LA	33	3.19
Wheeler Co., GA	Livingston Parish, LA	48	3.66
Wheeler Co., GA	Livingston Parish, LA	60	3.97
Madison Co., FL	Livingston Parish, LA	59	4.97
Madison Co., FL	Livingston Parish, LA	57	4.52
Madison Co., FL	Livingston Parish, LA	31	4.35
Marion Co., FL	Livingston Parish, LA	50	2.98
Walker Co., TX #1	Texas	30	3.38
Walker Co., TX #2	Texas	37	3.89
Trinity Co., TX #1	Texas	28	3.42
Trinity Co., TX #2	Texas	51	2.43
Madison Co., FL	Texas	36	2.96
Marion Co., FL	Texas	46	3.41

[a] Gall form is derived by dividing gall length by gall diameter. Round galls have low values; long galls have higher values.

stocked with trees from Livingston Parish, Louisiana, 6 plantings were Texas stock, and the remaining plantings were of more susceptible seed sources, i.e., southern Mississippi, northern Florida, and central Georgia. Plantings in Harrison County, Mississippi; Jackson County, Florida; Greene County, Georgia; and Marion County, Florida, were in adjacent blocks. All other plantings were separated by one to several miles. The length and width of 28 to 100 randomly selected galls were measured in each plantation. A gall form value (gall length/gall width) was determined for each gall. The means of the gall form values were used to compare plantations for the tendency of galls to be either long or round.

RESULTS AND DISCUSSION

Mean gall form varied among planting locations for all loblolly pine seed sources (Table 1). Plantings of seed sources from Mississippi, Georgia, and Florida had means above 3 and most were near 4. Gall form values varied for plantings of Texas stock, particularly in the state of Texas. Galls were observed to be predominantly round in the Trinity County, Texas, planting number 2, while galls in both the Walker County, Texas plantings and the other Trinity County site were longer and appeared to be shaped similarly to more susceptible pine populations. Results were much the same in Livingston Parish plantings, which had gall form values averaging from less than 3 in some plantings to near 5 in others. Low values for plantings in Early and Greene counties, Georgia, correspond with high resistance of Livingston Parish stock in these areas (H. Powers, Jr., personal communication). The high values for Livingston Parish stock in Madison County, Florida, suggest a breakdown of resistance at that location. This is consistent with Pait and Draper's findings (1983) that Livingston Parish stocks are highly susceptible to fusiform rust in Madison County, Florida.

The data support the hypothesis that fusiform rust galls tend to be round in resistant populations of loblolly pine.

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DISEASE RESISTANCE EVALUATION OF JACK PINE FOR WESTERN GALL RUST

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INTRODUCTION

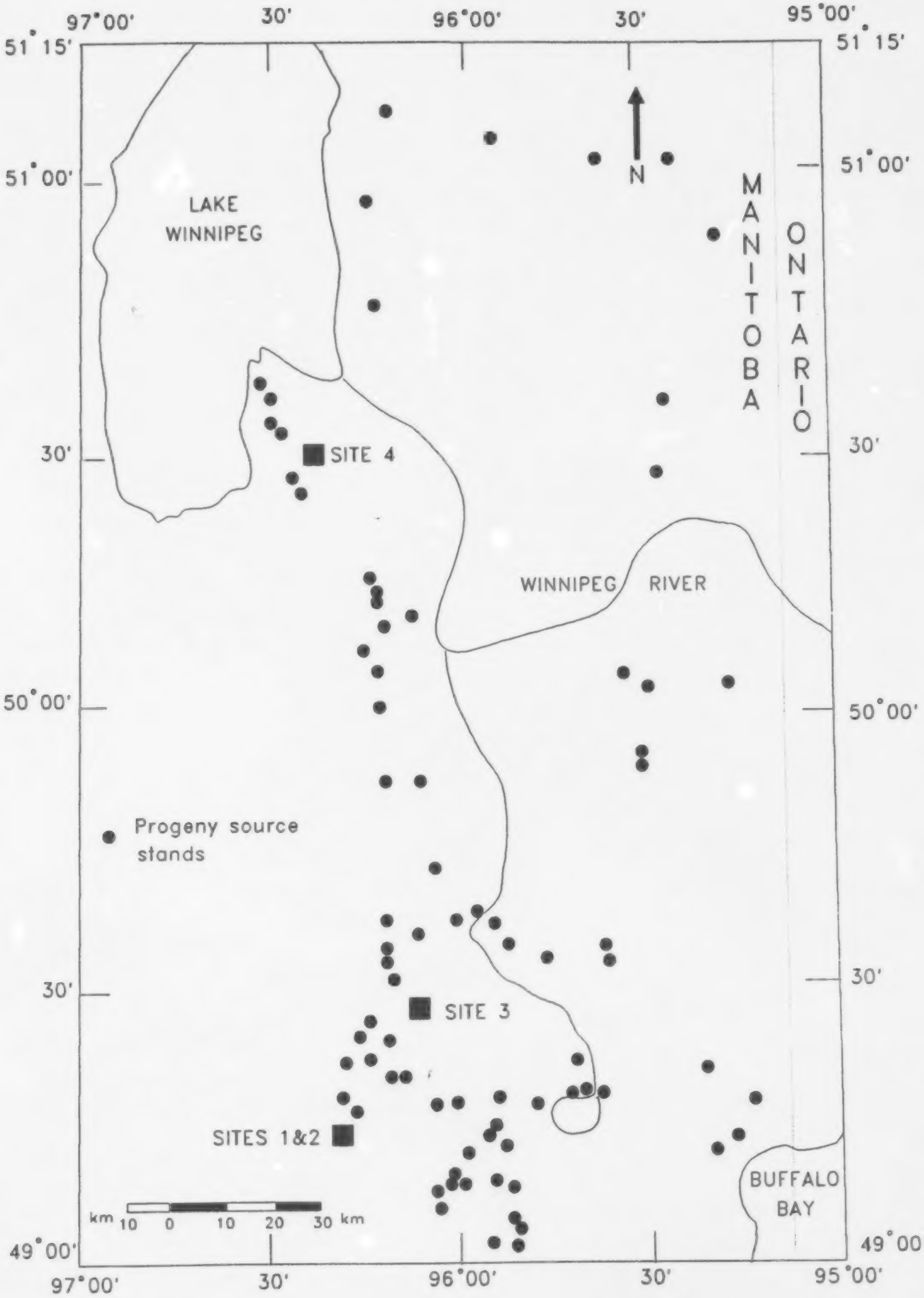
A program to develop genetically improved jack pine for Manitoba and Saskatchewan was initiated in 1967 by the Forestry Branch, Canada Department of Forestry and Rural Development, predecessor of Forestry Canada. Family tests consisting of replicated plantations of open-pollinated families were established in three areas in 1972, 1974, and 1976 to provide genetic quality information and breeding materials (Klein 1982). The test established in 1972 consisted of four plantations in eastern Manitoba. Each plantation had the same 209 open-pollinated families derived from wild parent trees selected in eastern Manitoba south of 51°15'N, six families from trees selected in Saskatchewan, and one bulked control lot from southeastern Manitoba (Fig. 1).

Western gall rust, caused by the rust fungus *Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka, has been observed in this plantation since shortly after planting. This disease is widespread on hard pines in Canada and is especially important in intensively managed stands (Bella and Navratil 1988; Hiratsuka 1981; Hiratsuka and Powell 1976; Powell and Hiratsuka 1973). Because of its potential impact on jack pine plantations, resistance to western gall rust infection should be included as a selection criterion in this breeding program, as in any hard pine breeding program in Canada (Yanchuk et al. 1988). An initial step in incorporating rust resistance into jack pine breeding is to develop and evaluate techniques for assessing host response to infection. This paper reports on assessment of host response to natural infection in family test plantations, and to greenhouse inoculation of seedlings with field-collected spores.

MATERIALS AND METHODS

Field Surveys

Assessment of response to infection under field conditions was done by recording occurrence of rust galls on all plot trees in four eastern Manitoba family test plantations at 17 years from planting (Fig. 1). Galls were counted on about 6400 trees in 216 family lots and 15 replications. Number of trees


97°00'
30'
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95°00'
51°15'
51°00'
30'
50°00'
30'
49°00'
LAKE WINNIPEG
N
MANITOBA
ONTARIO
SITE 4
WINNIPEG RIVER
Progeny source stands
SITE 3
SITES 1&2
BUFFALO BAY
km 10 0 10 20 30 km

Figure 1. Location of planting sites and source stands in Manitoba, Canada.

per family ranged from 19 to 31. Only the proportion of trees in each family with at least one gall was used in this report.

Greenhouse Inoculation

Seedlings used for the inoculation experiments were reared from seed produced by controlled matings between trees grown in the family test plantations. Forty open-pollinated families found to be genetically superior for growth and straightness were grouped into 20 pairs. More than one tree from each selected family was used in the mating to ensure adequate seed yield. Consequently, individuals from the same mating are related as first cousins rather than as full-sibs, and the seedlots are referred to as first-cousin families.

The main purpose of the matings was to produce stock for a seed orchard. Seed that was surplus to the requirements for the seed orchard was used for this study. This seed yielded from 6 to 129 seedlings per mating, with a total of 724 seedlings.

Seedlings of the 20 first-cousin families were reared in the Northern Forestry Centre greenhouse in Spencer-Lemaire "Five" Rootrainers, having a cavity volume of 55 cm^3, for 7 weeks prior to inoculation. A mixture of spores collected at several locations in central Manitoba was used for inoculation. Seedlings were sprayed with distilled water using an atomizer, then dry spores were applied to elongating epicotyl tissue with a soft paintbrush. Trays of inoculated seedlings were then incubated under a polyethylene sheet for several days. One month after inoculation the seedlings were transplanted into 4-L pots and were kept in the greenhouse under growth-promoting conditions through the observation period.

Seedlings were scored for visible reaction to inoculation in January (7 months after inoculation) and in May 1989 (11 months after inoculation). The rating scale, ranging from 0 for no symptoms to 5 for a complete gall, is illustrated in Figures 2 and 3. Mean score was calculated for each first-cousin family.

RESULTS

Field Survey

Percentage of trees in a family with at least one gall ranged from 0 to 79, with a mean of 25%, a standard deviation among families of 14%, and the standard error of a family mean of 1%. When family infection rates were grouped into frequency classes with an interval of 10%, the largest number of families was in the 21-30% class, and the frequency distribution was skewed to the right (Fig. 4).

Greenhouse Inoculation

Family mean scores in January ranged from 0.23 to 4.42, with a mean of 1.95, standard deviation among families of 1.08, and standard error of family mean of 0.24. Between the January and May assessments, some families changed in reaction scores within the 1.1-4.0 range, but there were no shifts from below 1.1 to above 4.0, indicating that satisfactory evaluation of resistance can be done 7 months after inoculation (Fig. 5).

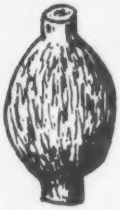
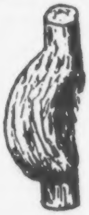
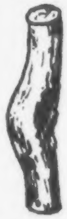
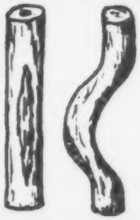
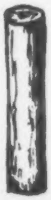
5		Complete gall, often with smooth surface
4		Partial gall, often with rough bark and necrotic canker
3		Some swelling with rough bark and open necrotic canker
2		Definite canker but no swelling
1		Visible discoloration or definite indication of infection such as acute bending of stem
0		No symptom

Figure 2. Evaluation criteria for jack pine seedlings for western gall rust.

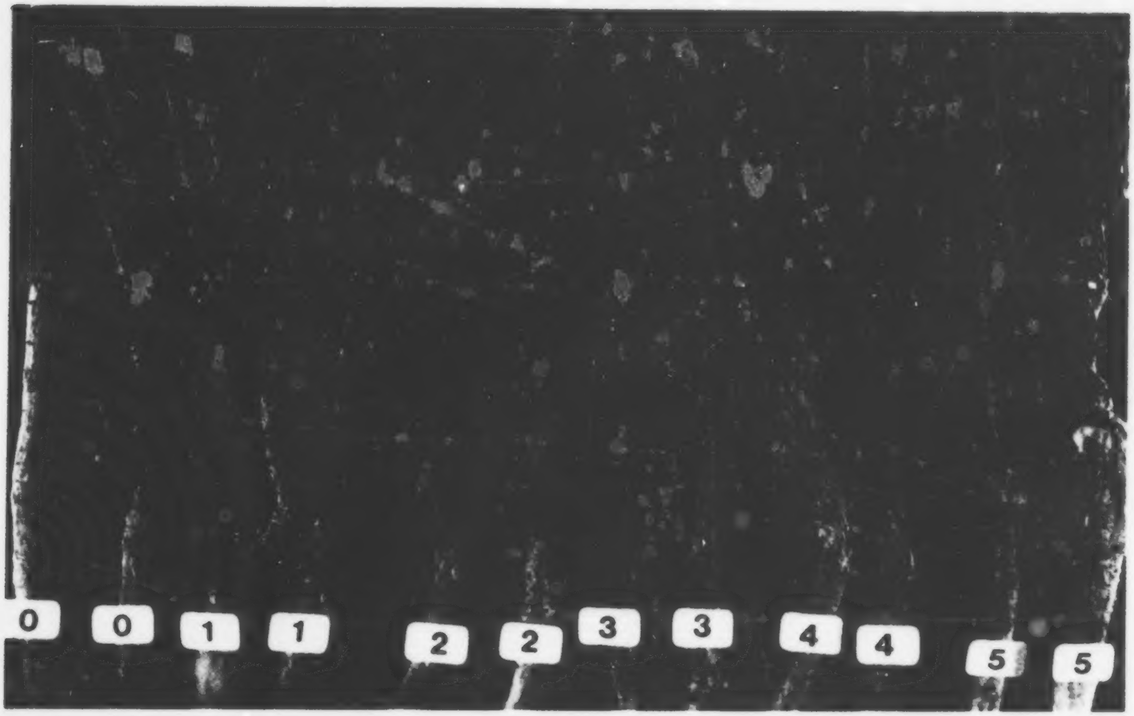


Figure 3. Jack pine seedings after 7 months of inoculation, with evaluation numbers.

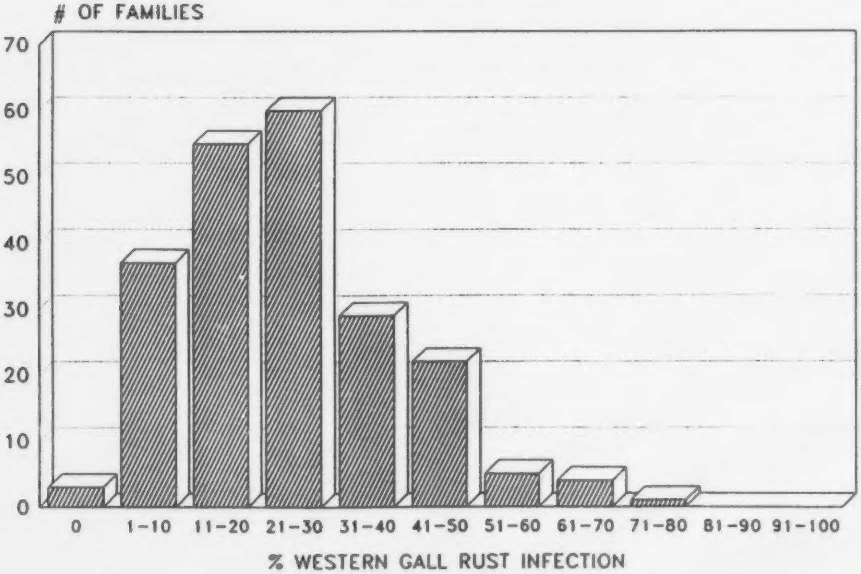


Figure 4. Incidence of western gall rust for the 216 families of 17-year-old jack pine.

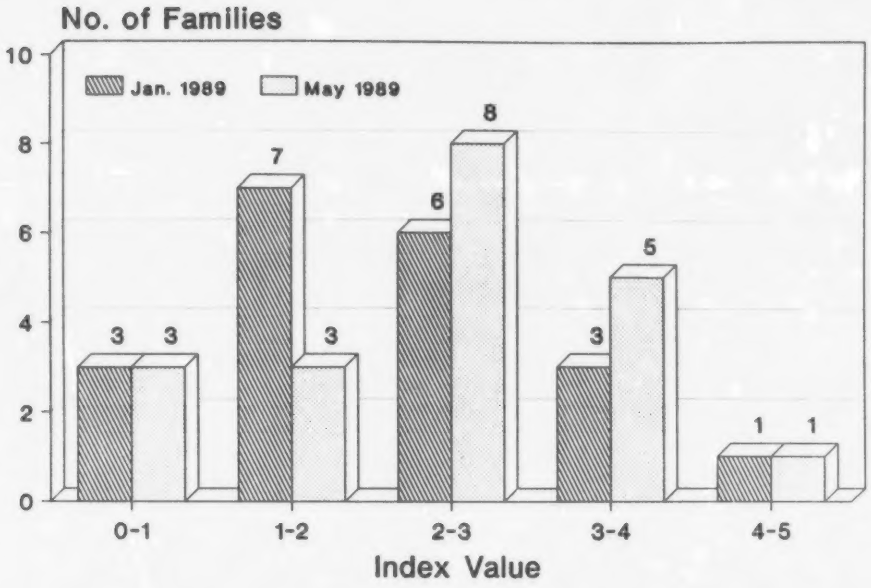


Figure 5. Shift of infection index values between evaluations after 7 months (January 1989) and 11 months (May 1989).

CONCLUSION

Field survey results from the family test plantations and results of greenhouse inoculation of first-cousin families show a wide range of family means, indicating the possibility of substantial genetic variation and the apparent effectiveness of assessment procedures used in these trials.

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PROGRESS IN DEVELOPING EARLY SCREENING TECHNIQUES FOR RESISTANCE OF PONDEROSA PINE TO WESTERN GALL RUST

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Variability in field resistance to western gall rust (WGR), caused by *Peridermium harknessii* J.P. Moore, has been noted in several pine species (Burnes et al. 1988; Hoff 1985; Merrill et al. 1986; Old et al. 1985; Thomas et al. 1984; Wenner and Merrill 1987; Yanchuk et al. 1988). Screening for resistance to WGR lags behind the notable systems for white pine blister rust (WPBR), caused by *Cronartium ribicola* J.C. Fischer ex Rabh. (Bingham 1983) and fusiform rust (FR), caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* (Powers 1984). Resistance screening studies for these latter diseases involve mainly field and greenhouse tests and, to a lesser extent, *in vitro* lab tests (Frampton et al. 1984). Several studies involving WGR have incorporated artificial inoculations in developing greenhouse screening systems (Blenis and Pinnell 1988; Burnes et al. 1988; Chen et al. 1988; Hoff 1985; Wenner and Merrill 1987). No study describing the interaction of *P. harknessii* with any host species under *in vitro* laboratory conditions has been published. However, *P. harknessii* has been grown in axenic culture (Allen et al. 1988), and systems to manipulate host tissues *in vitro* are increasingly available (Tuskan et al. 1990).

In this study, we have been examining various inoculation techniques for eventual use in genetic analyses of host-pathogen interactions. Specifically, several different host tissue systems have been inoculated to characterize reactions in *Pinus ponderosa* Dougl. ex Laws that may indicate resistance or susceptibility. For host material, we have used seedlings, excised embryos, host callus, and cell suspension cultures. For fungal inoculum, we have used aeciospores, excised pieces of gall tissue, and two vegetative forms of the fungus (Lundquist et al. 1990). Preliminary results of various inoculation techniques are described.

SEEDLING INOCULATIONS

The specific purposes of seedling inoculations were to determine 1) what pre-gall symptoms occur, how they develop with time, and which symptoms might prove useful as genetic markers of resistance, 2) what factors, besides resistance and susceptibility, alter symptom development and how these alterations could be quantified, and 3) whether pre-gall symptoms are correlated with subsequent galling. Seedlings (and embryos) were grown from a bulk seed lot collected at Denbigh Experimental Forest, Denbigh, North Dakota. Aeciospores used as inoculum in most trials were multiple gall (bulk) collections gathered in the spring of 1988 at Horning State Farm, Plattsmouth, Nebraska. Unless otherwise noted, suspensions of 25 mg aeciospores (Horning bulk) in 0.75 mL of oil (Soltrol 170, Phillips Petroleum Co.) were applied to each group of 96 25- to 30-day-old seedlings with an air pressure atomizer. Inoculated seedlings were placed into large black plastic bags lined with wet paper towels for 48 h at 20°C. The seedlings were taken out of the bag and left in a shaded, 20°C room for an additional 24 h before being placed in the greenhouse.

Initially, inoculated seedlings were surveyed for symptom development prior to gall formation. The following pre-gall symptoms were observed: red stem and needle pigmentation, needle death, adventitious shoot development, stem stunting, and seedling death. Red stem pigment was the first, most consistent, and most easily measured symptom. Red pigmentation also showed a characteristic pattern of development that could be quantified using disease progress curves. Generally, the curves showed a sharp increase in the number of pigmented seedlings 15-25 days after inoculation (dai) (Fig. 1). A plateau was reached between 30 and 50 dai, after which there was little further development.

The rate and magnitude of disease progress could be altered by varying several parameters. As inoculum load increased from 0 to 100 mg/mL of aeciospores, the rate and magnitude of symptom development also increased (Fig. 1A). Seedling age at the time of inoculation also influenced the shape of the curve (Fig. 1B). Seedling lots less than 12 days old or more than 85 days old had lower proportions of pigmented seedlings. Seedlings between 26 and 28 days old were most responsive. Aeciospores collected from seven individual galls from two locations (Nebraska and North Dakota) showed different disease progress curves (Fig. 1C). Inoculum grouped by location showed significant differences among locations (≤ 0.05). Finally, seedlings grown in very small containers (17 × 100 mm) with soil medium were more responsive than seedlings growing either in larger containers (25 × 250 mm) or in vermiculite (Fig. 1D).

Galls developed on seedling stems at about 80 dai. The proportion of seedlings showing pigment at 50 dai was correlated with the proportion of seedlings with galls at 120 dai. Stem pigmentation is not necessary for gall development; seedlings with and without pigment developed galls. The proportion of seedlings with galls increased until 250 dai. The correlation between the proportion of seedlings with pigment at 50 dai and the proportion with galls at 250 dai was nonsignificant.

EMBRYO INOCULATIONS

The specific purposes of embryo inoculations were to determine if embryos could be infected and develop early symptoms. Embryos were extracted from surface-sterilized germinating seeds, inoculated by brushing aeciospores onto their surface or by placing small pieces of vegetative cultures (white or orange mycelia) of *P. harknessii* onto the seedling surface, and placed into Petri plates lined with sterile moistened paper towels. Axenic cultures of *P. harknessii* were isolated as described elsewhere (Lundquist et al., these proceedings). Five dai embryo were planted into vermiculite-filled tubes, watered from below and incubated under grow lamps at 20°C. Subsequently, embryos were observed for macroscopic symptoms. None were found. Embryos were harvested 5, 10, and 20 dai and fixed in FAA. Ten embryos were observed at each of the nine inoculum type-inoculum time combinations. Embryos were embedded in paraffin, sectioned with a rotary microtome, stained with acid fuchsin, and observed under the light microscope. Haustoria and intercellular hyphae were often found in stem tissue inoculated with spores, seldom found in tissues inoculated with white mycelium, and not found with tissues inoculated with orange mycelium.

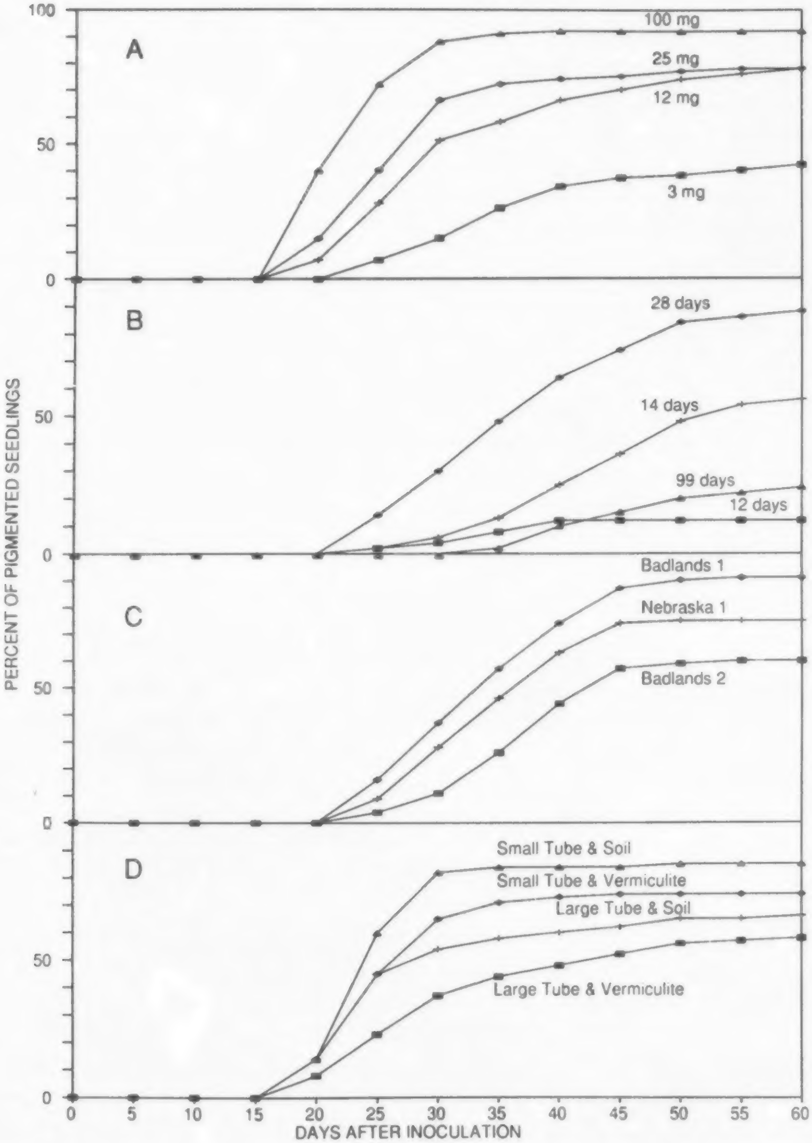

Figure 1. **Percent of seedlings developing stem pigment over time. A.** After inoculation with *P. harknessii* aeciospores at four inoculum densities (3, 12, 25, and 100 mg/mL). **B.** After being inoculated at four different ages (12, 14, 28, and 85 days old). **C.** After inoculation with *P. harknessii* aeciospores collected from three different sources. **D.** While growing in four different growth conditions of container size and soil type.

CALLUS INOCULATIONS

The purposes of callus inoculations were to determine if callus could be infected and develop early symptoms. Host callus was initiated by plating horizontal sections of surface-sterilized lateral buds collected in the spring from field resistant and susceptible 25-year-old *P. ponderosa* trees onto LS medium containing 1.0 mg NAA and 1.0 mg BAP per litre (Tuskan et al. 1990). Host callus cultures could be initiated and maintained for about 30 days, after which the callus turned brown. Buds were collected in spring 1989. At least one week prior to inoculation, host callus was transferred to fresh medium. For inoculations, aeciospores or pieces of axenic fungus colonies were placed on top of the callus or beside the callus in contact with callus and medium, or pieces of friable host callus were smeared into a mass of ungerminated spores.

When aeciospores were applied to the surface of host callus, spores usually germinated abundantly, but the germ tubes grew away from the host surface. Examination using light and electron microscopy showed no evidence of the fungus in the host tissue. When host callus was smeared on top of a mass of aeciospores, spores germinated, germ tubes made contact with individual host cells, but grew over their surface and on to the next cell. The fungus apparently had little effect on the health of the host, since most cells in the presence of the fungus showed no morphological change or uptake of the Evans Blue vital stain. In a few cases, the fungus apparently did penetrate host cells, in which case the host cell collapsed and Evans Blue was taken up. After 5 days under these conditions, host cultures were overrun with contaminants originating from the original aeciospore mix.

Examination of host callus initiated from previously infected gall tissue showed that *P. harknessii* could grow from the original explant into newly regenerated callus to form intercellular hyphae and haustoria. Such infected callus appeared morphologically similar to noninfected callus.

The possibility that the host produced a toxic chemical that diffused into the growth medium was tested by placing pieces of host callus into the middle of smeared cultures of *P. harknessii*. Callus from both resistant and susceptible selections caused *P. harknessii* colonies to turn brown after about 14 days. No statistical differences between selections were noted. The browning was attributed to a normal senescence reaction of the host.

DISCUSSION AND CONCLUSIONS

We approached this study on a broad front because it expanded the range of potential symptoms that might be used in a screening system. Although there were other early symptoms, stem pigmentation on seedlings was the most consistent early response. Prior studies have noted this pigmentation reaction to *P. harknessii* infection on *P. ponderosa* (Hoff 1985; Quick 1966) and other hosts (Allen and Hiratsuka 1985; Burnes et al. 1988; True 1938).

Disease progress curves are useful in quantifying pigment development over time and characterizing the influence of various factors on disease development. Parameters such as rate of disease increase and area under disease progress curves would offer traits useful in assessing the additive nature of disease resistance (Kinloch and Byler 1981). The present study indicated that rate and magnitude of the disease progress curve could be altered by several controllable factors. The effect of inoculum load on disease has been shown for *P. harknessii* on *P. contorta* Dougl. var. *latifolia* Engelm. (Blenis and Pinnell 1988); and the importance of carefully controlling inoculum loads in screening has been described for FR on southern pines (Griggs et al. 1984; Laird and Phelps 1975; Matthews et al. 1978; Matthews and

Rowan 1972). The effect of seedling age at the time of inoculation indicated a window of susceptibility to WGR in *P. ponderosa*, somewhat different than the increase in susceptibility with increase in age in FR and *Pinus taeda* L. (Rowan and Steinbeck 1977). The effect of WGR inoculum source has been mentioned by Chen et al. (1988) for *P. harknessii* on *Pinus radiata* D. Don, and has been studied for WPBR, rusts of hard pines in Italy, and FR (Powers 1982). The effect of soil conditions and container size has not been studied previously for WGR, but has been examined with WPBR (Kinloch 1980) and FR (Rowan 1977, 1978, 1979; Rowan and Steinbeck 1977).

Examination of correlations between stem pigment (and other early symptoms) and either resistance to or subsequent galling caused by FR has shown variable results (Kuhlman 1987, 1988; Lundquist et al. 1982; Walkinshaw 1986; Walkinshaw and Anderson 1987). With WGR on *P. contorta*, Allen and Hiratsuka (1985) found that only seedlings displaying red pigment ultimately developed galls, but not all pigmented seedlings developed galls. The results of the present experiment with *P. ponderosa* indicate that pigment is related to early gall development (before 120 dai), but not to subsequent galling. If this discrepancy could be resolved, stem pigment may prove to be a useful marker for early selection for WGR resistance.

Infected seedlings developed stem pigment, but embryos and callus did not. The inability to induce easily detectable symptoms in embryo and callus cultures and the inability to infect callus by applying inoculum present hurdles in the development of an *in vitro* screening system for WGR resistance. Advantages and disadvantages of *in vitro* selection schemes have been noted by various authors (Daub 1986; Frampton et al. 1983; Helgeson 1983; Ingram 1977; Maheshware et al. 1967; Miller and Maxwell 1983). Generally, control over the pathogen (e.g., genotype, inoculum density, time of inoculation), host (e.g., genotype, age, vigor), and environment is improved in *in vitro* systems. Furthermore, inoculated tissues can be monitored more closely. The *in vitro* technology for tissue culture of *P. ponderosa* and for axenic propagation of *P. harknessii* is not well developed, although these hurdles will probably be overcome. In contrast, basidiospores and vegetative cultures of both WPBR and FR have been used to infect embryos and cultured seedlings, and WPBR has been used to infect cell cultures. Although these studies seem to be aimed ultimately at establishing *in vitro* screening systems, apparently none have been put into operational use. These techniques, however, have created opportunities in the study of host-pathogen interactions. In this regard, we hope to characterize the physiology of the host-pathogen interaction.

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BEHAVIOR OF *CRONARTIUM FLACCIDUM* BASIDIOSPORES ON DIFFERENT ORGANS OF THE SAME PINE SPECIES

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Cronartium flaccidum (Alb. et Schw.) Wint., pathogenic agent of two-needled pine blister rust, noted in Italy by Di Micheli (1937) on *Pinus nigra* Arn. but present since 1830 (exsiccatae), has appeared in epidemic form since 1961 involving all the species of two-needled pine present in Italy: *Pinus nigra* with two provenances (Austrian and Abruzzo pines), *P. laricio* Poir. with two provenances (Calabrian and Corsican pines), *P. pinaster* Ait., *P. pinea* L., and *P. halepensis* Mill.

Contrarily, *P. sylvestris* L., precisely in the period of maximum diffusion of the pine blister rust (1962-1972), was marginally touched by the infection: 1964 Val Maira (CN) and 1970 Val Sarentino (BZ) (Moriondo 1975).

In the last 19 years, however, there have been no observed infections of *P. sylvestris*, whether in nature or in tests of artificial inoculation with suspension of basidiospores at different concentrations.

Within the research under way to isolate the possible mechanisms of resistance of the above-mentioned species, we have undertaken this work to determine the eventual susceptibility of Scots pine to the disease when inoculated at different phenological times and in comparison with a susceptible species, *P. pinea*.

MATERIALS AND METHODS

Seedlings of *P. sylvestris* and *P. pinea* were sprouted in plastic containers (20 per container, for a total of 200 seedlings per species) and placed in a climatic chamber, where they were inoculated respectively with blister rust basidiospores collected from Ospedaletto (Lucca) at 1200 m above sea level and from S. Rossore (Pisa) at sea level.

The attempt to induce infection was carried out on plants of Scots pine and Italian stone pine of 2, 8, and 24 months of age by hanging on the seedlings the leaves of *Vincetoxicum hirundinaria* Med. (intermediate host of blister rust), on which there was present a telial phase already formed for 3 days and, therefore, in full sporulation.

In the climatic chamber at a level of relative humidity between 90 and 95%, there was a corresponding temperature between 20 and 22°C. The analysis of the germinative power of basidiospores in agar water gave results of 74 and 78%, respectively, for the provenances of Ospedaletto and S. Rossore.

Starting 1 hour after inoculation and continuing in the following period, the cotyledons, and the primary and secondary needles were removed from 50 seedlings. In this way a total of 50 portions per organ was obtained to be treated according to the technique of fluorescence observation; the samples of needles were immersed for 10 min in a solution of 0.05% Calcofluor in distilled water. The observations were carried out on a Leitz Phloemopak 2.1 microscope with an incident light excitation system and equipped with UV filters and a 75-Watt Leitz 100z Xenon lamp (Ragazzi and Dellavalle Fedi 1982).

RESULTS

The percentage of seedlings infected in relation to the moment of inoculation (organ present) was 1.8% for *P. sylvestris* when the primary needles were present, while cotyledons and secondary needles did not respond.

Contrarily, for *P. pinea* the values were 98.8, 75.2, and 43.6% when there were present respectively primary needles, secondary needles, and cotyledons (Table 1).

The infection was considered to have taken hold in relation to the appearance of spots, which resulted at 83 days for primary needles of *P. sylvestris*; and at 55, 67, and 70 days, respectively, for primary needles, secondary needles, and cotyledons of *P. pinea*.

The average number of spots for seedlings was 4 for primary needles of Scots pine; and 42, 31, and 31 for primary needles, secondary needles, and cotyledons of Italian stone pine.

Table 1. Course of *C. flaccidum* infection on various organs of *P. sylvestris* and *P. pinea*

Organ	Total seedlings inoculated	Percentage seedlings infected	Spots		Basidiospore behavior[a]					
			Time of appearance (d)	Average number of seedlings	a	b	c	d	e	f
Cotyledons										
P. sylvestris	200	0.0	0	0	10.10	26	26.5	12.8	44	1.5
P. pinea	200	43.6	70	31	47.22	11	52.0	18.3	248	22.4
Primary needles										
P. sylvestris	200	1.8	83	4	25.60	16	33.0	16.1	115	6.4
P. pinea	200	98.8	55	42	87.30	5	46.0	78.0	400	70.0
Secondary needles										
P. sylvestris	200	0.0	0	0	17.60	20	32.2	14.2	46	2.2
P. pinea	200	75.2	67	31	68.10	8	70.0	31.0	301	61.0

[a] a) Percentage of germination, b) time of germination (hours), c) percentage of germinative tubes with one ramification, d) percentage of germinative tubes with more ramifications, e) length of germinative tube (μm) after 40 hours, f) percentage of germinative tubes that climb over the stomatic rim. Parameters have been considered on 50 portions per organ. Observations have been carried out considering 200 basidiospores per parameter.

The percentage of basidiospore germination appears different in relation to the infected organs: 25.6, 17.6, and 10.1% for primary needles, secondary needles, and cotyledons of *P. sylvestris*; 87.3, 68.1, and 47.2% for the same organs of *P. pinea*.

It is also interesting to note the difference between the lengths of germination time: 16, 20, and 26 hours for primary needles, secondary needles, and cotyledons of *P. sylvestris*; and 5, 8, and 11 hours for the same organs of *P. pinea*.

Between *P. sylvestris* and *P. pinea*, the data contrasts regarding the percentage of germinative tubes with only one ramification, the prevalent situation in *P. sylvestris* being 33, 32.2, and 26.5% for primary needles, secondary needles, and cotyledons; while on *P. pinea* the germinative tubes prevail with more ramifications: 78, 31, and 18.3%, respectively, for primary needles, secondary needles, and cotyledons.

Finally, also the data concerning the length of germinative tubes 40 hours after inoculation, and the percentage of tubes that climb over the stomatal rim, are in favor of the primary needles in respect to the others: 115, 46, and 44 µm for *P. sylvestris*, and 400, 301, and 248 µm for *P. pinea*; 6.4, 2.2, and 1.5% for *P. sylvestris*, and 70, 61, and 22.4% for *P. pinea*.

DISCUSSION AND CONCLUSION

The primary needles of *P. sylvestris* were shown to be the only organs susceptible to the infection of blister rust, while on *P. pinea* (a susceptible species), the secondary needles and cotyledons also became infected, if to a lesser degree. Moreover, the greater susceptibility of the herbaceous seedlings, in comparison to those plants of 1, 2, or more years, has already been demonstrated by Ragazzi and Moriondo (1979) through experiments on *P. nigra*; Raddi et al. (1979) for *P. pinea*; Patton (1961) and Patton and Riker (1966) for seedlings of *P. strobus* L. infected by *Cronartium ribicola* Fisch., about which Van Arsdel (1967b) has also verified the lesser susceptibility of the secondary needles of the same pine species. Bergdahl and French (1976a, 1976b), through the inoculation of *Pinus ponderosa* Laws. with *Cronartium comandrae* Pk., have observed how the susceptibility of the seedlings decreases with age; those of 1 month being more susceptible than those of 2 and 12 months. Miller and Cowling (1977) have also demonstrated for the binomial *Cronartium fusiforme* Hedg. and Hunt ex Cumm.--*Pinus elliottii* Engelm. that the susceptibility of the primary needles is clearly greater than that of the cotyledons and secondary needles.

The data concerning the behavior of basidiospores on different organs should be studied with close attention; in fact, the low percentage and the long time periods of germination certainly play against the microorganisms, considering that the basidiospores find the best conditions during the evening or the early morning hours, as shown both for *C. flaccidum* (Ragazzi and Ferrini 1978) and for other species of the genus *Cronartium* (Hirt 1935; Siggers 1947; Van Arsdel et al. 1956; Nighswander and Patton 1965; Powers 1966; Van Arsdel 1967a; Snow 1968; Snow and Froelich 1968; Snow et al. 1968).

The above-mentioned situation declines when the basidiospores are in the phase of germination when the temperature, the light intensity, and the low level of relative humidity can act as devitalizers.

Other situations unfavorable to *C. flaccidum* show up in the observation of the data concerning the presence of germinative tubes with one or more ramifications, and the lengths of the tubes themselves. Certainly longer tubes, but above all, those with more ramifications, can have greater probabilities of meeting with a stoma. Also, the percentage of germinative tubes that climb over a stoma, beyond the amount that effectively causes infection, is so low on *P. sylvestris* as to further reduce the probability of infection.

At the conclusion of this work, some considerations and reflections present themselves:

a) The greater susceptibility of the primary needles, documented bibliographically and verified experimentally, is such to have made possible the infecting process on a species held to be resistant (*P. sylvestris*).

b) From the findings, the impression asserts itself that the supposed resistance of *P. sylvestris* is not really such, but that the same species has escaped infection because of a series of circumstances:
   - the fact that the conditions were never optimal,
   - the fact that the epidemic of *C. flaccidum* always concerned old plantations, and
   - the fact that on the other species of pine the most devastating epidemics took hold on plantations or in artificial sowing, while *P. sylvestris* is abundant in the natural state.

c) It can be deduced, therefore, that to verify all the above it is necessary to correlate the given results (infection of the primary needles) with the exact phenological phase resulting in infection, and with the optimum concentrations of inoculum (basidiospores). In any case, this last is to be ascertained.

d) It is necessary to remember that in Scandinavia, northern Germany, Great Britain, and Holland, *P. sylvestris* is susceptible to pine-to-pine blister rust (*Endocronartium pini* (Pers.) Y. Hiratsuka), the aecial form of which is morphologically similar to *C. flaccidum* and which differs from this since it is a homothallic organism.

e) Finally, it ought to be underlined how, from this experience, it will become necessary in the near future, to verify which are the mechanisms pertaining to the pine needle that condition the various parameters we have considered, and that, as it appears, have diversified the response of the various organs of the plant to the disadvantage of the primary needles.

SUMMARY

Since 1961, the epidemics of *Cronartium flaccidum* blister rust of two-needled pine concerned, in Italy, all species of pine--*Pinus nigra* Arn. with two provenances (Austrian and Abruzzo pines), *P. laricio* Poir. with two provenances (Calabrian and Corsican pines), *P. pinaster* Ait., *P. pinea* L., and *P. halepensis* Mill.,--except *P. sylvestris* L., which appeared infected only in a few insignificant cases. In the last 19 years, *P. sylvestris* was not found to be infected either in nature or in tests of artificial inoculation.

The authors, in the course of research to recognize the possible mechanisms of resistance, looked for the susceptibility of different organs of the same Scots pine tree (resistant species) in comparison with those of an Italian stone pine tree (susceptible species). The inoculations were carried out on seedlings 2, 8, and 24 months old by hanging on them the leaves of *Vincetoxicum hirundinaria* Med. (intermediate host of the disease) on which there was present the telia phase.

Concerning *P. sylvestris* we noticed: 1) the susceptibility of only primary needles; 2) the consideration that the supposed resistance of *P. sylvestris* is not really such, but that the same species has escaped infection because of a series of circumstances; and 3) the necessity to correlate the susceptible phenological phase (primary needles) with the optimal concentration of inoculum (in any case, to be ascertained) to obtain infected material.

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THE EFFECT OF AGE ON THE SUSCEPTIBILITY TO BLISTER RUST OF WESTERN WHITE PINE SEEDLINGS

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INTRODUCTION

In British Columbia we have initiated a program to select western white pines (*Pinus monticola* D. Don.) that are resistant to white pine blister rust (*Cronartium ribicola* J.C. Fischer) (Meagher and Hunt 1986). Our program screens seedlings; it is similar to the programs in the U.S. Forest Service (Bingham 1983; Hunt 1988). Seedlings are 2 years old when inoculated, because younger *P. strobus* L. seedlings are highly susceptible (Clinton and McCormick 1919; York and Snell 1922) and it is believed that *P. monticola* behaves similarly (Bingham 1983). Likewise, if *P. strobus* is old when tested, it appears highly resistant compared to seedlings (Patton 1961, 1967). It is then possible that scions from highly resistant older *P. monticola* trees may produce very susceptible offspring. The objective in this study was to determine if *P. monticola* seedlings become more resistant with age and whether highly resistant older trees (screened as clones) produce susceptible offspring.

METHODS

In the first test, three seedlots from three different geographic areas (Sooke, Vancouver Island--1 tree; Barriére, Kamloops Forest Region--5 trees (pooled); and Kaslo, Nelson Forest Region--5 trees (pooled)) were grown for 4 successive years and then inoculated (Hunt 1988) at the same time. In the second test, open-pollinated seed was gathered from two highly resistant clones and a susceptible clone (Hunt and Meagher 1989). Infection incidence (needle spots and stem cankers) in offspring (average 51 individuals per seedlot) from these clones were compared to incidence in offspring from candidate trees, including a highly cankered control, and resistant Idaho F_2 (Bingham 1983), all inoculated at the same time.

RESULTS

Older seedlings averaged fewer spots per tree (Table 1) and had fewer trees with many spots compared to younger seedlings of the same seedlots. The 1-year-old seedlings had the fewest spots, because they were small targets, but on a per-metre basis they had 15-70 times as many spots as the older seedlings (Table 1). Spots were more frequent on the current foliage than on older foliage, and stem cankers originated only from spots on current foliage. Cankers were less frequent on 4-year-old seedlings than on younger seedlings.

The susceptible clone (A1) produced more cankered offspring than all other seedlots tested (Table 2). One of the resistant clones (G8) produced about as many spotted and cankered offspring as a control (C) candidate, while the other was about as resistant as Idaho F_2 stock (Table 2).

Table 1. **Blister rust spot and canker incidence in three seedlots (pooled) inoculated in 1987 at different ages**

Age (yr)	Sample size	Mean spots/tree	Mean[a] spots/m	Trees spotted (%)	Trees cankered (%)
1	113	8.7	11.70	100	100[b]
2	215	30.2	0.67	100	98
3	229	23.8	0.55	99	98
4	179	11.1	0.16	92	88

[a] Crown sizes of all trees were recorded, and two representative seedlings of ages 2, 3, and 4 years had the needles counted and measured to obtain a summed needle length representative for the age class. Needles were counted on all 1-year-old seedlings and summed for the seedlot, then seedlots were averaged.

[b] Most trees cankered about 5 months sooner than older seedlings.

Table 2. **Blister-rust incidence in offspring from resistant (r) and susceptible (s) clones, candidate trees, and Idaho F_2 orchard seed**

Seed parent	Spots/seedling (mean)	Trees cankered after 2 years (%)
s-A1	8.7	97
48	7.4	93
49	6.7	90
60	13.9	89
65	8.7	87
62	6.5	85
r-G8	8.5	83
C	8.7	81
48	8.7	79
65	9.4	79
r-B640	6.9	74
F2	4.3	73

DISCUSSION

Four-year-old seedlings become resistant to blister rust, showing fewer spots and cankers than their younger siblings. Cankering of 2- and 3-year-old seedlings was similar, although the latter had fewer spots (Table 1). One-year-old seedlings were very small targets, but developed relatively many more spots and cankers earlier than older seedlings. Like eastern white pine (Clinton and McCormick 1919; York and Snell 1922), 1-year-old seedlings are highly susceptible. The optimal age for rigorously screening seedlings is 2-3 years. For use in field sites of low-inoculum-density, perhaps older seedlings could be screened.

Since cankers were associated only with spots on current foliage, it must be concluded that under our inoculation conditions, all foliage except the current foliage is resistant to blister rust. This is contrary to observations of western white pine by Lachmund (1933) and eastern white pine by Hirt (1936, 1938) and to previous inoculation results of western white pine (Pierson and Buchanan 1938) where current needles are considered the most resistant. Only Snell (1936) and Patton (1961) report that current needles are most susceptible. More field inoculations are needed to confirm the high susceptibility of current needles and that cankers predominately originate from these needles; otherwise, results from screening seedlings may be an artifact of test conditions and not accurately reflect the field situation.

Resistant clones derived from screening old material as clones may produce susceptible offspring (Table 2); thus, this material is not suitable for seed orchards unless their offspring also indicate resistance.

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PERIDERMIUM PINI (PERS.) LEV.: AXENIC CULTURE AND INOCULATION OF PINE SEEDLINGS AND CALLUS CULTURES

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ABSTRACT

Axenic cultures of *Peridermium pini* have been established on modified Shenk and Hildebrandt's and Harvey and Grasham's media from naturally infected cortex tissues and aeciospore-infected calluses of *Pinus sylvestris* and from aeciospores collected from northeast Scotland and East Anglia. The cultures occasionally produced immature smooth-surfaced, binucleate spores. Axenic cultures were also obtained from 30-40% of single sporelings of some East Anglia spore sources, but not from northeast Scotland sporelings. Most colonies obtained from East Anglia spores had a smooth surface and clearly defined margin, while those from northeast Scotland sources predominantly had a fluffy surface and irregular margin. Seedlings of *P. sylvestris*, *P. nigra* var. *maritima*, and *P. mugo* vars. *mughus*, *rostrata*, and *pumilio* were inoculated at their cotyledon stage with aeciospores from northeast Scotland. Infections resulted in swelling, death of the seedlings, and formation of spermagonia after a year and aecia after 2 years. Callus tissue cultures of the host pines were infected by inoculation with northeast Scotland spores. With *Pinus radiata*, a nonhost, only intercellular hyphae were found, while with *Pseudotsuga menziesii*, another nonhost, infection was established much more slowly than with the host pines.

INTRODUCTION

Peridermium pini (Pers.) Lev. (*Endocronartium pini* (Pers.) Hiratsuka), an autoecious pine-to-pine rust fungus occurring in Europe, causes 'resin-top' disease of Scots pine (*Pinus sylvestris* L.). Corsican pine (*P. nigra* var. *maritima* (Aiton) Melville) and mountain pine (*P. mugo* var. *mughus* (Scopilo) Zenari) are also known to be its natural hosts (Wilson and Henderson 1966). Because *P. pini* is indistinguishable in its morphology from the aecial stage of the host-alternating rust, *Cronartium flaccidum* (Alb. and Schw.) Wint., it is regarded as a non-host alternating race of *C. flaccidum* (Wilson and Henderson 1966). This paper describes cultural studies on *P. pini* and experiments on the infection of very young pine seedlings and pine callus tissue.

P. PINI IN GREAT BRITIAN

In Great Britain, *P. pini* mainly occurs in northeast Scotland (Murray et al. 1969; van der Kamp 1970) and East Anglia (Gibbs et al. 1987; Greig 1987). On the basis of the cytology and germination of the aeciospores, Gibbs et al. (1988) proposed that the population of the fungus in Thetford, East Anglia, was different from that in northeast Scotland. Thus, the behavior of East Anglia spores conforms to the description of some European collections made by Hiratsuka (1968), i.e., spores germinate to form short, septate germ tubes and each cell of the germ tube has one nucleus. In comparison, spores from northeast Scotland produce long nonseptate germ tubes that frequently form vesicles at the tip. Two nuclei migrate along these germ tubes (Gibbs et al. 1988). A similar account of the germination behavior of aeciospores from northeast Scotland was provided by van der Kamp (1967).

Some new information on nuclear behavior of recently collected spores of *P. pini* from northeast Scotland is shown in Table 1. When the spores were germinated soon after collection, 70-90% of the germ tubes developed vesicles. The 18-h point for the determination of the number of nuclei was chosen as the one at which most vesicles had begun to form, and the 24-h point as the one at which many had developed a further extension--the vesicle tube. The main change that was noted was from a condition before vesicle formation where two nuclei were most commonly present to that after vesicle formation where the number had often increased to four. No observations were made that convincingly supported the idea of nuclear fusion followed by meiosis, although there was a slight increase in the occurrence of single nuclei after vesicle formation. Septa were commonly present in the vesicle tubes (at 96 h; 1% one-, 15% two-, 82% three-, and 1% four-septate).

CULTURAL STUDIES

Four pine stem rusts, *Cronartium fusiforme* (Hedgc. and Hunt) Cumm. (Hollis et al. 1972), *C. ribicola* (J.C. Fisch.) Rabenh. (Harvey and Grasham 1974), *C. quercuum* (Miyabe) Shirai (Yamazaki and Katsuya 1987), and *Endocronartium harknessii* (Moore) Hiratsuka (*Peridermium harknessii* Moore) (Allen et al. 1988) have now been grown away from their hosts. To establish axenic culture of *Peridermium pini*, a modified SH medium--1/4 SH basal medium (Shenk and Hildebrandt 1972) plus 3

Table 1. Number of nuclei in *Peridermium pini* from Northeast Scotland (0.5% water-agar, 20°C, HCl-Giemsa staining)

Incubation time (h)	Location of nuclei	No. of samples	No. of nuclei (%)					
			1	2	3	4	5	6
0	In ungerminated spores	225[a]	5	83	11	1	0	0
18	In germ tubes not forming vesicles	149	7	81	6	6	0	0
18	Only in vesicles	123	11	27	11	50	0	1
24	In vesicle tubes and associated vesicles	337	12	29	12	43	1	2

[a] Among them five three-nucleate spores attached together.

g/L 'Lab-Lemco' broth (Oxoid), 1 g/L malt extract, 2 mg/L kinetin, 0.5 mg/L 2,4-D, 30 g/L sucrose, and 8 g/L bacto agar--was used. Naturally infected cortex tissues of Scots pine from northeast Scotland were placed on the medium and after 8 weeks hyphae from 8% of the 'dual cultures' had colonized the agar. Axenic cultures were obtained by transferring these mycelia to a fresh medium[1].

The most efficient way of establishing axenic culture of *P. pini* proved to be direct inoculation of nutrient-agar medium with aeciospores[1]. Contaminant-free aeciospores could be obtained from unbroken aecia, and these aecia could be kept in a satisfactory condition for up to one year at −12°C. By this means, cultures have been established from aeciospores collected from both northeast Scotland and East Anglia[1].

Fungal colonies grown on agar consisted of two types of hyphae: long, straight ones (type A) and twisted, corkscrew-like ones (type B). Both types of hyphae were mononucleate and a pair of electron-dense diaphragms were present in both sides of the septal pore[1].

Cultures of *P. pini* very occasionally produced immature spores. These spores were irregularly shaped and with a smooth surface. Both spores and spore-proliferating hyphae were predominantly binucleate, and the proportion of binucleate spores was similar to that recorded in naturally formed aeciospores by Gibbs et al. (1988).

In an attempt to establish axenic cultures of *P. pini* from single spores, three spore sources from East Anglia and three from northeast Scotland were tested[2]. The surface of the medium was cut into grid pattern and sparsely inoculated with aeciospores by using a sterile camel hair brush. Single sporelings were then mapped on graph paper. In 6 weeks, 27-38% of the single sporelings of two East Anglia sources developed into transferable and subculturable colonies. By contrast, none of the single sporelings of northeast Scotland sources formed recognizable colonies.

In another experiment described in detail by Pei and Gibbs[3], six spore sources from East Anglia and six from northeast Scotland (including all those used in the experiment on single spore culture) were seeded at a density of 1000-5000 spores/cm^2. All formed transferable and subculturable colonies. Six weeks after transfer from the original plates (incubated for 5-6 weeks after spore inoculation) to the SH medium, more than 87% of the colonies from East Anglia spores had formed cream-colored stromata with mucilaginous or occasionally felty surfaces. They comprised only B-type hyphae and had clearly defined margins. By contrast, 94% of colonies from northeast Scotland spores were white and fluffy. They comprised both A and B hyphae and had irregular margins.

INOCULATION OF YOUNG SEEDLINGS

In contrast to the situation with *Cronartium fusiforme* (Jewell 1960), *C. ribicola* (Kinloch and Comstock 1980), and *Endocronartium harknessii* (Allen and Hiratsuka 1985), there are no reports of the successful infection of very young seedlings with *P. pini*. In June 1987, seedlings of Scots pine, Corsican pine, and three varieties of mountain pine (*Pinus mugo* vars. *mughus* (Scopoli) Zenari, *rostrata* Hoopes., and *pumilio* (Haenke) Zenari) were inoculated at their cotyledon stage with aeciospores from northeast Scotland[4]. When the pines were examined by hand-sectioning 6 months after inoculation, incidence of infection was 35% in Scots pine, 50% in Corsican pine, 35% in *P. mugo* var. *mughus*, 45% in *P. mugo* var. *pumilio*, and 15% in *P. mugo* var. *rostrata*. Of 133 inoculated Scots pine seedlings, 43 showed swelling and 21 produced spermogonia (15 accompanied with swelling, the others not) in August 1988. In late April to early May 1989, 23 months after inoculation, aecia were produced on 19 seedlings, all of which had previously formed spermagonia. In a second experiment, infection of Scots pine seedlings from seven sources in the UK was determined 6 weeks after inoculation by stem-sectioning. Some 30-70% of the seedlings were infected by *P. pini*. The appearance of discoloration and necrosis on cotyledons and primary needles during the period was not always related to stem infection.

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[1] Pei, M.H.; Pawsey, R.G. Axenic culture of *Peridermium pini*. (In preparation.)

[2] Pei, M.H.; Gibbs, J.N. Axenic culture of *Peridermium pini* from single spores. (In preparation.)

[3] Pei, M.H.; Gibbs, J.N. Cultural characteristics of *Peridermium pini* from Northeast Scotland and East Anglia. (In preparation.)

The presence of spermagonia in infected seedlings is consistent with other records for *P. pini* from northeast Scotland (van der Kamp 1969; Olembo 1971). The fact that aecia only developed on those seedlings that produced spermagonia is intriguing and might suggest that a sexual process is occurring. However, such a view is not really supported by the evidence on nuclear behavior in the germ tubes as described earlier. It may also be noted that spermagonia have never been recorded in trees infected with the East Anglia form of the fungus and yet aecia are produced in abundance (Gibbs unpublished).

INOCULATION OF PINE CALLUS TISSUES

Hitherto, only two pine stem rust fungi have been used in inoculation experiments on callus cultures. Harvey and Grasham (1969, 1970) successfully infected callus tissues of *Pinus monticola* Dougl. with aerial mycelium and basidiospores of *Cronartium ribicola*. The mycelium derived from host-rust cultures even infected calluses of a nonhost species, *Pseudotsuga menziesii* (Mirb.) Franco (Harvey and Grasham 1971). However, another pine blister rust, *C. fusiforme*, did not establish typical infections in callus cultures of its hosts when inoculated with mycelium or basidiospores (Hare 1978; Jacobi et al. 1982).

Embryos of Scots pine, Corsican pine and the three varieties of mountain pine cited above were isolated and inoculated onto MS medium (Murashige and Skoog 1962) containing 1.5 mg/L kinetin, 0.5 mg/L 2,4-D (2,4-dichlorophenoxyacetic acid), 30 g/L sucrose and 8 g/L bacto agar. The callus cultures were incubated at 25°C in the dark and transferred at intervals of 4 weeks. They were inoculated with aeciospores collected from northeast Scotland by dusting with an aseptic camel hair brush. After 2 weeks, aerial hyphae started to grow from some of the calluses and eventually 11-64% of the pine calluses were colonized by *P. pini* (Pei 1989). Infections were characterized by the formation of aerial hyphae on the callus surface and intercellular hyphae and typical haustoria in the callus tissues. Hyphae from some of the infected calluses grew into the medium and axenic cultures were obtained from these mycelia.

When embryo-derived calluses of two nonhosts, *Pinus radiata* D. Don and *Pseudotsuga menziesii* were inoculated with aeciospores from northeast Scotland, aerial hyphae developed from 15 out of 35 uncontaminated calluses of *Pinus radiata* and 7 out of 17 *Pseudotsuga menziesii*. However, growth of the aerial hyphae was usually confined to a limited area on calluses of *Pinus radiata* and was less vigorous on *Pseudotsuga menziesii* than that on Scots pine calluses. Only intercellular hyphae were found in the aerial hyphae-carrying calluses of both *Pinus radiata* and *Pseudotsuga menziesii* when examined by sectioning (with *Pseudotsuga menziesii*, examination was conducted on samples dissected from parts of the calluses) 12 weeks after inoculation. During further incubation, calluses of *Pinus radiata* continued to grow actively and, within 5 months, most had become free of fungal hyphae (the original sites at which hyphae were present having either become necrotic or having become overgrown by newly formed callus). Calluses of *Pseudotsuga menziesii* showed only slow growth and four had died within 5 months. The other three were covered by fungal hyphae and both haustoria and intercellular hyphae were present in these calluses. The hyphae had also penetrated the surrounding medium.

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[4] Pei, M.H.; Brodie, J. Inoculation of young pine seedlings with aeciospores of *Peridermium pini*. (In preparation.)

In another experiment, 5-week-old Scots pine seedlings were inoculated with aeciospores from northeast Scotland in June 1987. Seven months later, after the presence of infection had been determined by stem sectioning, calluses were grown from four infected seedlings and four uninfected seedlings by plating surface-sterilized, *P. pini*-free needle fragments on MS medium. These were inoculated with aeciospores from northeast Scotland and fungal colonization was determined by the formation of aerial hyphae. After 31 of the 160 calluses had been discarded because of contamination, 43% of the calluses from uninfected and 34% from infected seedlings were colonized by *P. pini*. This difference was not statistically significant. Part of the explanation for this could lie in physiological changes occurring in the infected seedlings, which could have led to some induction of resistance in the calluses. More critical work in this area is clearly required.

CONCLUSION

Axenic cultures of *P. pini* have been obtained from naturally infected Scots pine cortex, inoculated callus, and aeciospores. There were differences between spore sources from East Anglia and those from northeast Scotland in culturability of single spores and in culture morphology. On the basis of the work carried out on this and other pine stem rust fungi, it is clear that comparative studies on many aspects of these biotrophs using axenic cultures are now feasible. In particular, the application to the cultures of techniques of molecular biology should lead to a better understanding of the nature of variation among and within species of this group of rusts. The possibility of using cultures derived from single spores is especially intriguing.

Young pine seedlings and callus tissue cultures have been infected by inoculation with aeciospores of *P. pini*. The successful inoculation of cotyledons and calluses makes it possible for easily handled plant materials to be used in studies of hard pine-*P. pini* interactions. It is also likely that effective seedling screening could provide a practical way of obtaining *P. pini*-resistant trees in the future.

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INOCULATING LODGEPOLE PINE WITH *ENDOCRONARTIUM HARKNESSII*

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ABSTRACT

Reliable inoculation techniques are valuable for epidemiology and resistance studies. Three methods for inoculating lodgepole pine with *Endocronartium harknessii* were compared. Teliospores diluted with oil, water, or talc were each used to inoculate 120 seedlings in eight experiments. A chromatography sprayer was used to apply the oil- and water-suspended spores. An apparatus for applying the talc-suspended spores was developed. Oil caused some phytotoxicity and resulted in 60% infection. The other two methods caused no phytotoxicity and were virtually identical in terms of spore deposition, spore germination *in situ*, and seedling infection (84%). Any of the three inoculation methods might be used in resistance studies; but the talc method applies spores in a dry state, simulating spore dispersal in the field, and thus might be preferable for epidemiological studies.

INTRODUCTION

Western gall rust, caused by the fungus *Endocronartium harknessii* (J.P. Moore) Y. Hiratsuka, is the most destructive stem rust of hard pines in western Canada (Ziller 1974), and thus there is considerable interest in the epidemiology and host-parasite relations of this rust. In infection studies, it is important to have a reliable inoculation method. A number of different methods have been used to inoculate pines with western gall rust. For example, undiluted spores were dusted onto the surface of prewetted lodgepole pine seedlings, which then were wrapped in wet paper towels and enclosed between polyethylene sheets (Allen and Hiratsuka 1985). Another method (Nelson 1972, Quick 1966) involved spraying trees with spores suspended in water. Following inoculation, the seedlings were placed inside a polyethylene tent, covered by a canvas tent. Intermittent misting was then done to maintain free water on the surface of the seedlings. A modification of this method, using a polyethylene box as an inoculation chamber, was developed in our laboratory and recently described (Blenis and Hiratsuka 1986). A disadvantage with the use of water-suspended spores, however, is that the teliospores of *E. harknessii* are quite hydrophobic and thus do not form a uniform and stable suspension in water or in a solution of water and Tween (Blenis, unpublished). Spores tend to rise to the surface when mixed with water, even following vigorous shaking of the mixture. Two other possible inoculation techniques would be to use oil or talc as diluents. Light mineral oil has been used to inoculate jack pine (*Pinus banksiana* Lamb.) with western gall rust (Burnes et al. 1988). Talc, which has been used as a spore diluent for *E. harknessii* (Hoff 1985), has some advantages. Such a mixture is more stable than spores suspended in water, would be less likely than oil to react with the host cuticle, and would likely result in a more uniform deposit of spores than if they were applied without any diluent. For epidemiological studies, the use of dry spores might be more representative of the natural phenomenon in which spores are disseminated in a dry state and later encounter water at the infection site.

The primary objective of this research was to compare three methods using talc, water, and oil as diluents for inoculating lodgepole pine (*P. contorta* Dougl. var. *latifolia* Engelm.) seedlings with *E.*

harknessii teliospores. The criteria for comparison were, phytotoxicity, spore deposition, spore germination, and seedling infection.

MATERIALS AND METHODS

Spores

E. harknessii spores were collected near Hinton, Alberta. They were passed through a 45 µm mesh sieve to remove debris, and put into open 10-mL vials which were kept in a desiccator containing $CaCl_2$ at approximately 4°C, for 1-5 days until they could be transferred into 2-mL vials and stored in liquid nitrogen. Most vials contained a mixture of spores from four to ten galls. Prior to use, the vials of spores were warmed for 5 min in a 40°C water bath. Viability of the spores used in each experiment was determined by germinating them on 1.5% water agar for 24 h.

Seedlings

Eight inoculation experiments were conducted. Seedlings for the first four experiments were grown inside a greenhouse in Fives Rootrainers (Spencer-Lemaire Ind. Ltd.) in peat moss and fertilized biweekly with 200-200-200 ppm of N-P-K. The temperature was about 22°C, and the photoperiod was 18 h. The third and fourth experiments sustained some aphid damage. Consequently, seedlings in the last four experiments were grown inside a growth room. Conditions were similar to those for the first four experiments with the following exceptions: fertilizer containing 229-29-154 ppm of N-P-K plus 5.5 ppm of iron (Carlson 1979) was applied biweekly, the pH of the peat moss was adjusted to 5-5.5, and the temperature was 25°C during the simulated day and 18°C during the simulated night. Following inoculation, seedlings were maintained under the greenhouse conditions described above.

Inoculation Experiments

In each experiment, three methods referred to as the oil, water, and talc methods were used to inoculate two trays of 60 seedlings each, aged 9-11 weeks. Inoculum concentrations were chosen, based on preliminary tests, with the aim of obtaining equal spore deposition with the three treatments. In the oil treatment, 15 mg of spores were suspended in 10 mL of Soltrol 170 mineral oil (Phillips 66 Co., Chicago IL) and sprayed onto the trees with a glass chromatography sprayer. Within 1 min, the trees were sprayed with 20 mL of deionized water. In the water treatment, 32 mg of spores were placed in 40 mL of water, agitated by hand, and sprayed with a chromatography sprayer onto the seedlings. For the talc treatment, 12 mg of spores were mixed with 0.48 g of talc. Initially, a cyclone separator (Tervet 1951) was evaluated for dispersing this mixture, but it failed to produce a uniform spore cloud. Therefore, a new method was developed. Spores were placed into an inverted pipette tip attached to a vibrator. Activation of the vibrator shook the spores into an air stream passing underneath, which dispersed the spores onto the seedlings (Blenis and Pinnell 1988). Within 5 min of inoculation, the trees were sprayed with 40 mL of water. Controls consisted of 20 seedlings inoculated with each of the diluents alone, except for the talc treatment in which the seedlings were merely sprayed with water before incubation. Following inoculation, a wire screen was placed over each individual tray, wet paper towels were placed over the screens, and the trays were put inside opaque polyethylene bags which were then sealed and put in a dark incubator at 17°C for 24 h. To maintain high humidity during the incubation period, the trays had been immersed in water to the depth of the soil line prior to inoculation.

Spore deposition on 1-cm epicotyl segments was measured with a dissecting microscope at 100 × on 15 seedlings per treatment per experiment. Spore germination *in situ* was determined by selecting eight seedlings from each of the three treatments in three of the experiments. After removal of needles, epicotyl segments were vapor-fixed with OsO_4, air-dried for 2 days, mounted on stubs, sputter coated, and examined with a scanning electron microscope (SEM). Attempts were made to examine 30 spores per sample, although some specimens had fewer. Seedling infection was evaluated 5 months after inoculation. Analysis of variance (ANOVA) was used to compare the effect of inoculation method on seedling infection, following arcsine transformation of the infection frequencies to stabilize variance (Snedecor and Cochran 1967).

RESULTS

Germination of spores on water agar plates was between 75% and 89%, except for one experiment (not used in the SEM study) in which germination was 57%.

The oil treatment caused some phytotoxicity in the first four experiments. No trees were killed, although some were stunted and showed a reddening of needles within a few days of inoculation. There was much less impact of oil in the last four experiments. Water and talc had no effect on the trees.

ANOVA indicated that inoculum method had no effect on the coefficients of variation (CVs) of spore deposition (Table 1) obtained in the eight experiments. The talc method appeared to result in more spores being deposited per centimetre of seedling tissue, despite our intention of obtaining equal amounts of deposition with the three methods. Germ tube morphology on the surface of the seedlings, as seen with SEM, was not affected by diluent. Spore germination, however, appeared to be reduced by the oil treatment (Table 1), although lack of homogeneity of variances even following arcsine transformation (Snedecor and Cochran 1967) precluded use of ANOVA.

The average percentages of seedlings infected were 60%, 84%, and 84% for the oil, talc, and water treatments respectively (Table 1). ANOVA indicated that the diluent effect was significant at $p = 0.01$ (Table 2). Post hoc comparisons indicated that there was no significant difference in infection frequency between the water and talc techniques. The oil technique, however, resulted in significantly fewer infections.

DISCUSSION

All three methods easily could be scaled-up for mass testing of seedlings. Because each tray is contained in its own high-humidity environment, any unit that can maintain a temperature of 17°C for 24 h can serve as an incubation chamber. The technique that used oil as the diluent caused some phytotoxicity and resulted in less seedling infection than the other two methods, although these problems might be overcome by using less oil and more spores. For example, no phytotoxicity occurred when 30 seedlings were sprayed with 1.5 mL of oil (Burnes et al. 1988). Although the spores formed a more uniform suspension in oil than water, this method did not result in greater uniformity in spore deposition on the seedlings. Furthermore, of the three methods, it likely least simulates the method of spore dispersion that occurs under field conditions.

The water and talc methods were equally good in terms of phytotoxicity, variability of spore deposition, spore germination, and seedling infection. With the exception of experiments 3 and 4, which

Table 1. Effect of spore application method on the amount and variation of spore deposition, spore germination, and seedling infection

Method	Spores/cm[a]	CV[b]	% spore germination[c]	CV[d]	Infection	
					Avg. (%)[e]	Range (%)[f]
Oil	32	70	31	74	59.6 (64.5)	39-79 (52-79)
Talc	101	60	57	43	83.8 (91.3)	55-96 (89-96)
Water	58	76	52	16	84.0 (89.2)	64-99 (81-99)

[a] Number of spores observed on 1 cm of seedling epicotyl. Averaged over 15 seedlings for each of eight experiments per treatment.

[b] Average of the eight CVs of spore deposition (one per experiment).

[c] Percentage of germinated spores on the seedling surface. Averaged over eight seedlings for each of three experiments per treatment.

[d] Average of the three CVs of spore deposition (one per experiment).

[e] The numbers in brackets are based on exclusion of two experiments with aphid damage.

[f] The numbers in brackets are based on exclusion of two experiments with aphid damage.

Table 2. ANOVA table[a] for the effects of experiment, inoculation method, and method × experiment interaction, on the arcsine transformed values of percentage of seedling infection

Source	Sum of squares	Degrees of freedom	Mean square	F-ratio
Experiment	2146.4	7	306.6	9.1[b]
Method	4286.7	2	2143.3	28.8[b]
Rep × method	1040.7	14	74.4	2.2[c]
Error	809.1	24	33.7	

[a] A mixed model was used with method as a fixed variable and experiment as a random variable. The error term used for the method effect was the method × experiment mean square; the error term for the experiment effect was the error mean square.

[b] Significant at $p = 0.01$.

[c] Significant at $p = 0.05$.

sustained aphid damage, both methods consistently resulted in infection frequencies greater than 80% (Table 1).

The spores of some dry-spored fungi such as rusts, smuts, powdery mildews, and some hyphomycetes typically may not be dispersed suspended in water droplets. Inasmuch as suspending these spores in water may influence their germination behavior, it may be desirable in epidemiological studies to have a method for inoculating with dry spores. Settling towers have been used for this purpose, but for studies with gall rust on pine seedlings, the vertical orientation of the susceptible epicotyl would require extremely large amounts of spores to ensure adequate spore deposition. The apparatus for dispersing spores and talc described in this paper was simple to construct, and when used in conjunction with the incubation procedures described, reliably resulted in seedling infection. This apparatus now is routinely being used in our laboratory.

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A STUDY ON THE BLISTER RUST OF *PINUS MASSONIANA* OF CHINA

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ABSTRACT

Blister rust of *Pinus massoniana* is a serious disease. The pathogenic fungus is identified as *Cronartium flaccidum* (Alb. et Schw.) Wint. f. sp. *siphonostegium* Jing et Wang. Its telial host is *Siphonostegia chinensis* Benth. The incidence of the disease is higher in artificial young stands of *P. massoniana* and in stands on hill ridges. The aecia appear in early April. The duration of aeciospore dispersal is 51 days, with the peak period from April 26 to May 20. The spores can be caught at a 30-m distance and a 5-m height. Optimum conditions for aeciospore germination are high relative humidity, 23°C, and pH 7. The urediniospores start to spread in early May. The peak is on May 22. The optimum temperature for urediniospore germination is 25°C. The spores germinate best on a 2% agar water membrane with added extract of *S. chinensis*. The telia are formed in the second or last 10 days of May. The teliospores germinate in the same year. *S. chinensis* may be killed with a saturated salt solution. The killing effect on *S. chinensis* is not satusfactory with 76% $C_{12}H_{14}O_3C1_2$. Smearing a solution of 500× Topsin and $C_6H_7O_3NS$ and 500× Triadimefon on the surface of the diseased stem gives better results.

INTRODUCTION

The blister rusts of pine are very serious diseases. Up to now there have been many studies on the diseases in China (Jing et al. 1986; Ju et al. 1984; Liu et al. 1986; Shao et al. 1977; Shi 1984; Tong 1981) and abroad (Arthur 1962; Grand et al. 1972; Hiratsuka 1968, 1971, 1976; Peterson 1973; Ziller 1974). Only we have studied the blister rust of *Pinus massoniana* in recent years (Jing and Wang 1985, 1988). *P. massoniana* Lamb. is widely distributed in 15 provinces and autonomous regions; and it is an important timber and green tree species. With more extensive planting of this species, the blister rust of *P. massoniana* is spreading in Shaanxi, Sichuan, Hubei, and Guizhou provinces. In recent years, the disease has become very serious in Ankang and Hanzhong prefectures of Shaanxi. The average development rate is 14.7% in a 777-ha stand; the highest is 87.3%. The infection index is 49.4. The blister rust has caused great damage to forest production and continues to spread. To understand the pathogen, alternate host, infection cycle, disease development process, and control measures, a systematic study of the disease has been made since the beginning of 1984.

CONDITIONS OF DISEASE DEVELOPMENT

From 1984 to 1986, 17 sample stands were set up in 11 forest farms in Ankang County. One hundred sample trees were investigated in each stand. The average disease development rate was 14.7%, the highest was 87.3%; the disease index was 7.8, the highest was 49.4.

Investigation of Disease Development

It was found that there is a close relationship between the blister rust of *P. massoniana* and slope position, stand age, and distribution of understory alternate host plants (Table 1). The infection index is larger in 6-year-old stands than in 10-year-old ones. The most serious disease occurs in the stem 20 cm off the ground and in stands on ridges and upper slopes. This is because occurrence of the blister rust is related to distribution of the alternate host plant. The more alternate host plants, the more pathogen, and the more serious the disease is.

Damage of the Disease to Trees

Infection by the disease decreased height and diameter growth and needle length and damaged phloem of the *P. massoniana*. Growth of the main stem point and lateral branches of diseased trees was reduced by 35.6% and 33.7%. The dry weight of new branches was reduced by 67.8% (Table 2).

SYMPTOMS OF THE DISEASE AND PATHOGENIC FUNGUS

Symptoms of the Disease

The disease occurs on tree bark of the main stem and branches. In the Ankang region, light red-brown spermogonial nectar was produced in the last 10 days of August. The color turned red-brown, like a bloodstain, when dried. There was slight swelling in the diseased area. Cracks appeared on diseased bark between the last 10 days of March and the beginning of April in the 2nd year. Yellow blister aecia emerged from the cracks. After 4 to 7 days the aecia broke and aeciospores spread out. Then the diseased bark became rough. Because the resin passage was damaged, resin flowed out and condensed into a light yellow or grey-black tumorlike object.

The disease occurred mainly on the lower part of the stem and on the bottom of lateral branches. It may develop every year and have significant influence on tree growth. Diseased trees are subject to damage by *Dioryctria splendidella* Herrich-Sch. and *Monochamus alternatus* Hope. If the disease spreads around the stem, the tree will die (Fig. 1).

Alternate Host of the Pathogenic Fungus

Through several years of investigation, uredinia and telia were found on leaf stalks, undersurfaces of leaves, tender stems, sepals and fruit of *Siphonostegia chinensis* Benth. under natural conditions of Ankang County. To identify the relationship between *S. chinensis* and the disease, artificial inoculation experiments were made outdoors and indoors on 13 species such as *S. chinensis* Benth., *Paeonia lactiflora* Pall., *Ribes tenue* Jancz., *Pedicularis resupinata* L., and *Paeonia obovata* Maxim. which are possible alternate host plants of the disease fungus, during 1984 to 1986 at the Xiangxitong Experimental Farm in Ankang. The disease developed only on *S. chinensis* (Table 3). The incubation period was 6 to 10 days. Symptoms of the disease, and the shapes of uredinia, telia, and teliospores were the same as those developed in natural conditions. Therefore, it may be concluded that the alternate host plant of the pathogenic fungus is *S. chinensis*.

Table 1. Disease development of blister rust

Sample	Altitude (m)	Tree age (years)	Slope position	Stand	No. trees	No. diseased trees	Rate of infection (%)	Disease index	No. *S. chinensis* per m^2
1	500	6	Ridge	Mixed	121	87	71.9	38.4	4.2
2	350	9	Ridge	Mixed	321	36	11.2	5.9	1.5
3	550	13	Upper	Pure	210	13	6.2	3.1	1.0
4	480	10	Ridge	Pure	269	28	10.4	3.7	2.1
5	400	6	Upper	Pure	113	39	34.5	15.5	1.7
6	480	6	Ridge	Pure	212	185	87.3	49.4	3.9
7	520	8	Upper	Pure	160	13	8.1	4.5	2.0
8	470	7	Ridge	Pure	113	17	15.0	7.1	2.4
9	400	10	Mid	Pure	110	7	6.4	3.0	0.2
10	380	8	Lower	Pure	120	5	4.2	2.0	0.0

Table 2. Influence of blister rust on tree growth

Item	Total length of main branches	Total length of lateral branches	Total weight of new branches	Dry weight of new branches	Needle length on 1-year-old tree
Healthy tree	1024.0 m	796.2 m	37.35 kg	23.59 kg	13.6 cm
Diseased tree	659.0 m	528.2 m	29.59 kg	7.59 kg	10.3 cm
Reduced	35.6%	33.7%	20.8%	67.8%	24.3%

Table 3. Results of artificial inoculation with aeciospores

Plants tested	No. of leaves incubated		Results		Incubation period (days)	Appearance of telia (days)
	Upper	Lower	Upper	Lower		
Siphonostegia chinensis Benth.	77	394	-[a]	+++[b]	6-7	20-25
Paeonia obovata Maxim.	18	83	-	-	-	-
P. lactiflora Pall.	78	175	-	-	-	-
P. suffruticosa Andr.	11	24	-	-	-	-
Impatiens balsamina L.	87	118	-	-	-	-
Cynanchum atratum Bunge.	3	14	-	-	-	-
Pedicularis resupinata L.	26	142	-	-	-	-
P. lineata Franch. ex Maxim.	38	79	-	-	-	-
Artemisia capillaris Thunb.	39	39	-	-	-	-
Trifolium vulgare Ness.	-	26	-	-	-	-
Tropaeolum majus L.	4	7	-	-	-	-
Ribes tenue Jancz.	34	64	-	-	-	-
Carpesium cernuum L.	-	24	-	-	-	-

[a] - means no incidence.

[b] +++ means many uredia and telia appeared.

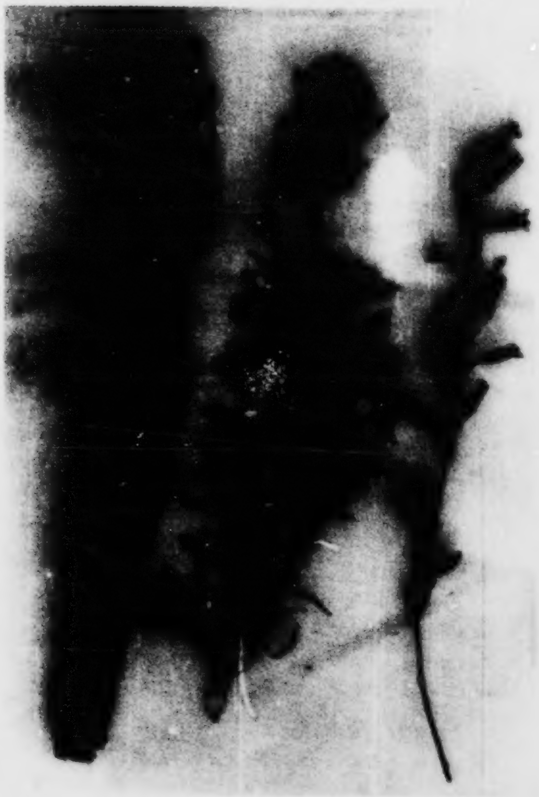

Figure 1. **Symptoms of the blister rust of *P. massoniana* and its alternate host *S. chinensis* Benth.**

Description of the Pathogenic Fungus

Spermogonia are flat or irregular in shape underneath the bark of *P. massoniana* and spermatia are like pears, colorless and transparent, 1.9-3.6 × 1.5-2.7 μm. Spermogonial sterigmata are closely arranged like a palisade. Aecia are like blisters, irregularly shaped, orange, and grow out of the tree bark 1.1-1.3 mm. Peridia of aecia are light yellow-white and composed of two or three layers of cells. Aeciospores are orange, globose or ellipse-shaped, 18.8-26.3 × 16.3-20.0 μm. On the netlike grainy surface of aeciospores there are many tower-shaped verrucae. Each verruca has five to seven annuli. The two annuli at the base of the verruca are very thick. There are fibrils among the verrucae (Fig. 2).

Uredinia appeared on the undersurface of leaves of *S. chinensis* at the beginning of May. At first, they are light green shiny spots containing fat; later they become small, light yellow blisters; then the blisters break and turn into yellow powder. Urediniospores are round or oval, 15-21.3 × 12.5-16.3 μm, and bright yellow. There are many tapered thorns bent like hooks on the top (Fig. 3). Hair-like telia appear around or in the middle of uredinia; they are upright or bent, red-brown, and 604-785 μm long. Teliospores in the telia are the same size (Fig. 4), spindle-shaped, with a smooth surface, and 27.5-36.5

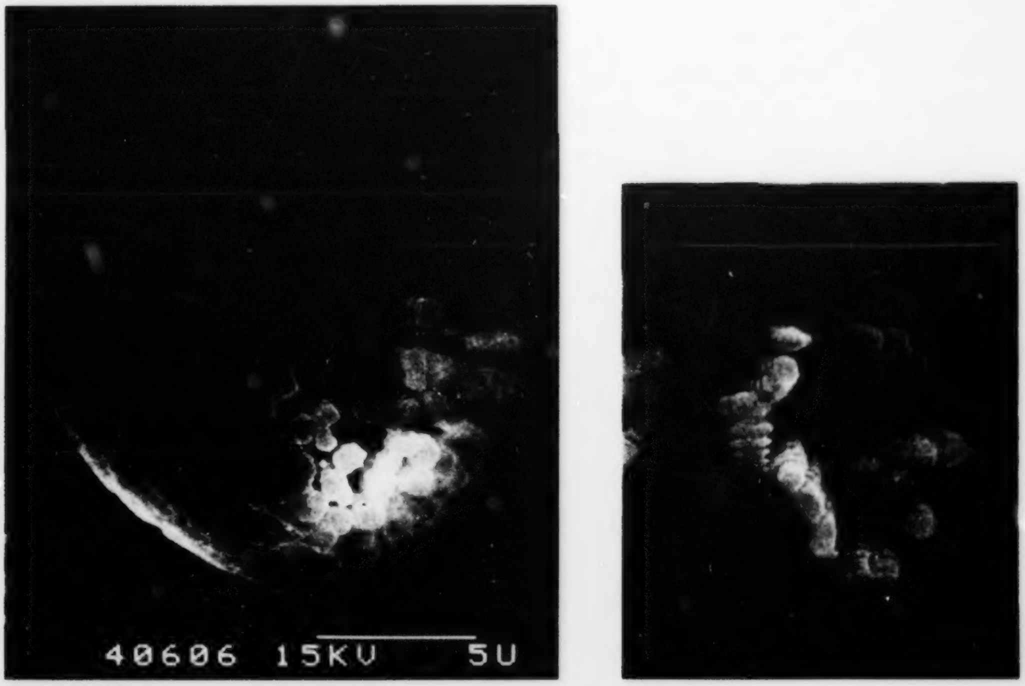

Figure 2. Aeciospore (× 50 000). **a.** Surface. **b.** Tower-shaped verrucae showing annuli.

× 12.5-16.3 μm. The cell wall is 0.9-1.6 μm thick. Mature teliospores may produce basidia and basidiospores when moistened. Basidia are bent (Fig. 5), and basidiospores are oval or kidney-shaped, 8-11 μm long, and colorless.

Cytological Characteristics of the Pathogenic Fungus

Each mature aeciospore shows two nuclei per cell when stained with HCl-Giemsa. Aeciospores may germinate in 2 h, and inner protoplasm enters the germ tube in 4 to 6 h. Two cell nuclei move towards the distal end of the germ tube in 8 to 10 h and the germ tube produces branches during growth; but no isolation membrane is produced, and no nuclear division was observed.

There is one nucleus in each cell of the mycelium of bark diseased by the blister rust. A monokaryotic mycelium grows in strands among the cells of the host plant. It may produce bar-like or thread-like haustoria that enter the host cell to absorb nutrients. The host cell nucleus will disintegrate soon after the haustoria contact it.

Identification of the Pathogenic Fungus

Aeciospores and urediniospores of the blister rust are basically the same as those on *Pinus sylvestris* L., *P. sylvestris* var. *mongolica* Litvin., and *P. tabulaeformis* Carr. (northeastern China). But

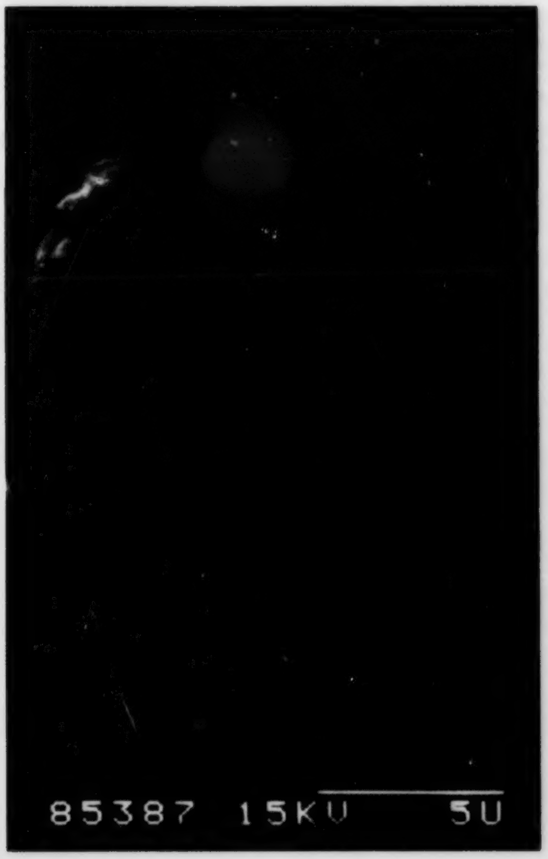

Figure 3. Urediniospore surface (× 5200).

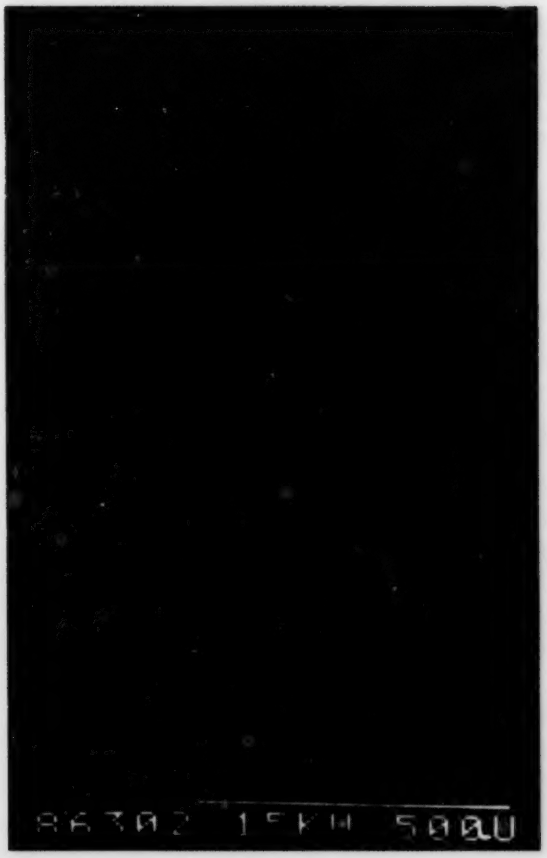

Figure 4. Telia on sepal of *S. chinensis* (× 90).

there are some significant differences: there is no obvious smooth surface on the aeciospores; the two annuli at the base of the tower-shaped verrucae are very thick; the telia are very small; their lengths are seldom beyond 800 μm; and the teliospores on the upper and lower parts of the telia are the same size and shape. Based on many artificial inoculation experiments made in 3 years, the disease fungus infects only *S. chinensis*, and does not infect *Paeonia obovata* Maxim., *Paeonia lactiflora* Pall., or *Pedicularis* spp. So the pathogenic fungus of the blister rust of *P. massoniana* is identified as *Cronartium flaccidum* (Alb. et Schw.) Wint. f. sp. *siphonostegium* Jing et Wang.

SPREAD AND GERMINATION CHARACTERISTICS OF THE SPORE

Spreading Time, Horizontal and Vertical Distances

In 1984 and 1985, 20 spore traps were set up 0.5 m above the ground in the forest on April 2. Observations were made once daily. The results showed that aeciospores began to spread from April 8 to June 5. The peak period of aeciospore spread was from April 26 to May 20. The aeciospores caught

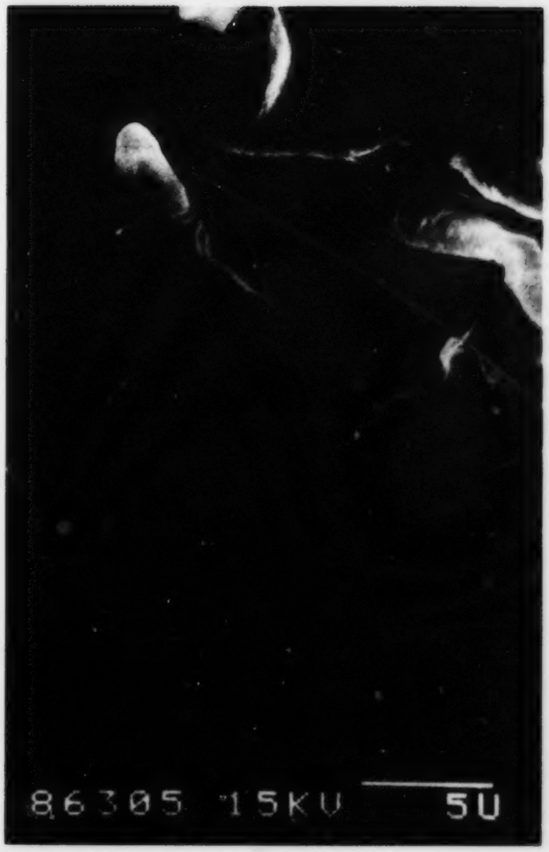

Figure 5. Teliospore that has germinated and produced basidia and basidiospores (× 3800).

during the period accounted for 81.5% of the total amount caught. Urediniospore dispersal began in early May and ended in the last 10 days of August. The peak period of spreading was after May 22.

During the peak period, seven traps were made at distances of 2, 5, 10, 20, 30, 40, and 50 m away from the diseased tree. At the same time, a pole was erected 2 m away from the diseased tree. Six more traps were attached to the pole at 1-, 2-, 3-, 4-, 5-, and 10-m heights. The catch results showed that horizontal spreading distance is 30 m. The most spores were found in the trap at 10 m; no spores were found in the catcher 50 m away. Spores were found in all six spore traps at different heights, but most spores were found in the traps lower than 3 m.

Spore Germination Characteristics and Measurement of Vitality

Germination of Aeciospores

The optimum temperature for germination is 20-24°C (Fig. 1). The optimum relative humidity is above 90%. Spores suspended in water rarely germinate. The spores germinate best on 2% water agar with extract of *S. chinensis*. The spores may germinate in liquid of pH 3-11, but the best is pH 7. The extract of *S. chinensis* can stimulate spores to germinate and raise the germination rate.

Germination of Urediniospores

The optimum conditions for urediniospore germination are temperature 25°C, relative humidity 90%, pH 7, and diffused light.

Germination of Teliospores

Diseased leaves of *S. chinensis* with telia were sampled on May 24, put in culture dishes with wet absorbent cotton and held at 22-26°C for 48 h. Spores began to germinate after 3 h, and 45% of the total spores germinated after 10 h.

Measurement of Spore Vitality

Mature aeciospores were put into a test tube covered with a cotton plug, and the tube was kept at 20°C under scattered light. The germination rate was measured every two days. The results showed that the spores can still germinate after 18 days, but the germination rate is very low, only 1.2%. After 20 days, spores cannot germinate. The vitality of urediospores is longer; 2-15% of urediospores on *S. chinensis* overwintering outdoors may germinate and have the ability to infect through inoculation.

INFECTION CYCLE OF THE DISEASE

Spermatial nectar appeared in cracks of the bark. Aecia came out from the lower-layer tissues of spermogonia at the end of March to the middle of May the following year. The peridium broke after aeciospores were mature. Aeciospores were spread by wind to the leaves and tender stems of *S. chinensis* and invaded through the stoma after germination. Uredinia appeared on the undersurface of leaves 6 to 10 days later. Urediniospores were spread by wind and could infect leaf, tender stem, sepal, and fruit of *S. chinensis* again. Telia were formed from the end of May to the middle of August. Mature teliospores may germinate to produce basidia and basidiospores in the same year. Basidiospores were spread by wind to needles of *P. massoniana*, then germinated and invaded through the stomata. Because aecia were found on the 2-year-old branches in the investigation, spermogonia had been formed on the stem in one to three years. The infection cycle of the disease is shown in Figure 2.

PREVENTION AND CONTROL OF THE DISEASE

Selection of Chemical Fungicide

Seven kinds of fungicides and 21 different concentrations were used in the experiment. Different fungicide solutions were sprayed on 2% agar plates containing aeciospores. Three repetitions were made of each concentration and compared with sterile water. Under 20°C and constant high humidity, the aeciospores were cultured for 24 h. A germ tube inhibition experiment was done. Aeciospores on the 2% agar plates germinated, 24 h later were sprayed with the fungicides, then cultured another 24 h. If the color of germ tubes changed from transparent to opaque, and the internal substances were concentrated, separated, broken, and diminished, it meant that the aeciospores were killed. Examination under microscope showed that all fungicides and concentrations have different inhibition effects on spore germination and germ tubes. Among these, 400× sodium p-aminobenzene sulfonate and 50% 500× thiophanate solution gave the best results.

Prevention and Control Experiment in the Forest

An experiment was done in two forest farms in Ankang County in the beginning of April. The experimental stands were 5- to 6-year-old *P. massoniana* forest. Seven kinds of fungicides and 20 concentrations were used. Water and diesel oil were used as solvents. The fungicide solution was smeared on the diseased stems. Two observations were made--one in the middle of May and another a year later. The criteria of effectiveness were: aecia did not break; aecia withered; or, even if the aecia broke, the aeciospores could not spread. The results are shown in Table 4.

It can be seen from Table 4 that diesel solutions of 500× 50% Topsin, 25% Triadimefon, or 80% Zineb, and kerosene solution of 400× sodium p-aminobenzene sulfonate were effective. In the year of treatment, inhibition rate was greater than 90%. No disease symptoms were found the following year. Diesel oil also had some killing effect on the pathogenic fungus.

Experiment on Killing Alternate Host Plants

In May of 1985 and 1986, an experiment was done in Ankang by spraying saturated salt solution or 2,4-D ($C_{12}H_{14}O_3Cl_2$) on the leaves of *S. chinensis*, and watering with the solutions. Observations were made 5 days later. Table 5 shows that saturated and 20% salt solutions were effective, but the 2,4-D solutions were not.

Killing alternate host plants is not practical in the forest, but it is possible to kill *S. chinensis* around the nursery by spraying with saturated salt solution.

ACKNOWLEDGMENT

Chen Dexiang of the Ankang Forest Research Institute and students Chen Hui, Chen Yuming, Yang Lin, and Shi Jianning of the Northwestern College of Forestry also took part in this investigation.

Table 4. Results of smearing fungicide on the diseased stem

Fungicide	Concentration	Solvent	No. trees treated	Current effect (%)	No. diseased trees next year
50% Topsin	2000×	Water	5	80.0	-[a]
	1000×	Water	5	86.7	1
	500×	Diesel	5	93.3	0
25% Triadimefon	1000×	Water	5	86.0	3
	800×	Water	5	90.0	1
	500×	Diesel	5	93.8	0
80% Zineb	1500×	Water	5	83.3	3
	1000×	Water	5	86.7	2
	500×	Diesel	5	93.0	0
75% Amobam	300×	Water	3	72.2	2
	200×	Water	3	83.0	1
	150×	Diesel	3	88.9	1
$C_6H_7O_3NS$	1000×	Water	5	70.0	5
	800×	Water	5	83.3	3
	500×	Diesel	5	90.0	0
Sodium p-aminobenzene sulfonate	800×	Water	10	73.8	-
	400×	Kerosene	10	90.9	-
Carboxin	1600×	Water	10	68.3	-
	800×	Water	10	78.1	-
	400×	Kerosene	10	93.1	-

[a] - means no investigation.

Table 5. Effect of salt solution and $C_{12}H_{14}O_3Cl_2$ on *S. chinensis*

Solution	Concentration	No. sprayed	No. killed	Death rate (%)
Salt	Saturated	115	105	91.3
	20%	121	88	72.7
	15%	116	68	58.6
$C_{12}H_{14}O_3Cl_2$	1000×	60	35	58.3
	2000×	44	22	50.0
	3000×	26	9	34.6
	4000×	46	15	32.6
Water	-	45	0	0.0

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SUSCEPTIBILITY OF ASIAN AND AMERICAN OAKS AND PINES TO *CRONARTIUM QUERCUUM*

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INTRODUCTION

Various forms of the pine-oak rust, caused by *Cronartium quercuum* (Berk.) Miyabe, are native to North America and eastern Asia. Several studies have been made on the host preferences of this rust in both areas (Hedgcock and Siggers 1949; Kondo 1975). Although the rust alternates between various species of hard pines and oaks in all areas, there are discrete differences not only between the North American and Asian forms, but also between the rusts occurring on the various pine hosts in North America. First and foremost is a striking difference in the time of infection of the pine host. In the Orient, infection of pine most commonly takes place from mid-September to mid-October. In the United States, infection takes place in May and June, depending upon the location. The galls produced on the Asian pine by *C. quercuum* are ovoid or globose, while in North America the gall shape depends upon the pine species involved. On *Pinus banksiana* Lamb. and *P. virginiana* Mill., for example, the galls are globose, while on *P. taeda* L. and *P. elliottii* Engelm. var. *elliottii* the galls are fusoid or spindle-shaped. Kaneko et al. (1990) have compared morphological aspects of basidiospores from Asia and North America. There is some evidence to indicate the existence of two strains of the rust in Japan: a *P. densiflora-Quercus serrata* strain and a *P. thunbergii-Q. acutissima* strain (Kondo 1975). However, the *thunbergii* strain was not available for our studies.

MATERIALS AND METHODS

Researchers in Japan, Korea, and the USA inoculated as many pine and oak species as possible with rust inoculum only from their own country. We did not want to risk introduction of the rust from the other countries. The original plan was to use *Quercus rubra* L., *Q. nigra* L., and *Q. phellos* L. from North America, along with *Q. serrata* Ziebold and Zucc. and *Q. acutissima* Carruth. from Japan and Korea. However, we also were able to obtain acorns of *Q. mongolica* (Fisch.) var. *grosseserrata* (Blume) Rehd. and E.H. Wils. and *Q. aliena* Blume from the Orient for the inoculations in Athens, Georgia. Pine species to be inoculated were *P. taeda*, *P. elliottii* var. *elliotti*, *P. virginiana*, *P. banksiana*, and *P. echinata* Mill. from the United States, along with *P. densiflora* Sieb. et Zucc. and *P. thunbergii* Parl. from the Orient. Each group inoculated each of these pine and oak hosts with their respective rust fungi. In the United States, we used aeciospores of the four *formae speciales* of *C. quercuum*: *fusiforme* from *P. taeda* and *P. elliottii* var. *elliottii*, *virginianae* from *P. virginiana*, *banksianae* from *P. banksiana*, and *echinatae* from *P. echinata*. In Japan and Korea, the rust spores were primarily from *P. densiflora*, the Japanese red pine.

RESULTS

Although relatively few oaks were available for inoculations in Athens, and the aeciospores of f. spp. *banksianae* and *echinatae* had low viability, uredinia or telia or both occurred on all but two host-pathogen combinations (Table 1). Infection on *Q. rubra* was very heavy after inoculation with either the *fusiforme* or *virginianae* forms of the rust. *Quercus mongolica* var. *grosseserrata* had somewhat less infection. These results indicate that both Asian and North American oak species are susceptible to infection by aeciospores of the four North American strains of *C. quercuum*.

In Japan, Dr. Kaneko included a larger number of oaks, and found that three of the North American oaks, *Q. rubra*, *Q. nigra*, and *Q. phellos*, were somewhat susceptible to inoculum from *P. densiflora* (Table 2). In some cases, for example, with the *Q. nigra*, North American oaks were as susceptible as the most susceptible of the Japanese oaks, which were *Q. serrata* and *Q. mongolica* var. *grosseserrata*. The Japanese isolates of the rust did not form uredinia on the American oaks, but did form them on the Japanese oaks. Thirty days after inoculation, no telia occurred on the American oak species, but some were developing on *Q. serrata*. However, after 75 days, the telial formation on *Q. nigra* was as frequent as that on *Q. serrata*. In contrast, inoculations with the four *formae speciales* from North American oaks usually produce uredinia in 10-14 days, and telia within 21 days. The inoculations of North American oaks with the Japanese isolates of the rust gave indications of some interaction between the oak host and the rust fungus that slows the development of the fungus on American oak seedlings for at least 30 days. This is a very significant difference between the Japanese collections of the rust and those in the United States. Oak inoculations in Korea, in contrast to those in Japan, produced very heavy infection on both *Q. rubra* and *Q. serrata*. The fact that *Q. rubra* was heavily infected with the Korean form of *C. quercuum*, while the Japanese form produced only light infection, indicates that further study is needed to determine if there is a strain difference between the two geographic sources of the rust.

In Athens, we inoculated *P. taeda*, *P. elliottii* var. *elliottii*, *P. virginiana*, *P. densiflora*, and *P. thunbergii* with *C. quercuum* f. sp. *fusiforme* and f. sp. *virginianae* when the pine seedlings were 1 and 4 months old (Table 3). The results with *fusiforme* were as we anticipated, in that *P. taeda* and *P. elliottii* were quite susceptible, but *P. virginiana*, *P. densiflora*, and *P. thunbergii* were rarely infected when 1-month-old seedlings were inoculated. When the seedlings were 4 months old at inoculation, the percent

Table 1. **Infection of five species of *Quercus* with four *formae speciales* of *Cronartium quercuum* from the United States**

Quercus species	*Cronartium quercuum* f. sp. *fusiforme*	*virginianae*	*banksianae*	*echinatae*
rubra	UT***(3/3)[a]	UT***(3/3)	UT*(3/3)	UT**(3/3)
mongolica var. *grosseserrata*	UT**(4/5)	UT***(5/5)	UT*(4/5)	UT*(4/4)
acutissima	UT*(2/4)	UT*(3/5)	T*(1/4)	UT*(2/3)
serrata	T*(2/3)	UT*(1/4)	T*(1/2)	--(1)
aliena	T**(1/1)	UT*(1/1)	T*(1/1)	--(1)

[a] UT indicates that either uredinia, telia, or both, were formed; ***, **, or * indicate heavy, medium, or light infection, respectively; parentheses surround number with indicated response per total number of plants.

Table 2. **Results of oak inoculations in Japan with aeciospores of *Cronartium quercuum* from *Pinus densiflora***

Quercus species	Number of seedlings inoculated	% seedlings infected 15 days after inoculation Fleck	II	III	% seedlings infected 30 days after inoculation Fleck	II	II & III	III	% seedlings infected 75 days after inoculation III
nigra	29	14	0	0	28	0	0	0	34
phellos	21	10	0	0	10	0	0	0	24
rubra	20	5	0	0	5	0	0	0	10
acutissima	8	0	0	0	0	0	0	0	0
mongolica var. *grosseserrata*	8	0	38	0	0	38	0	0	5
serrata	8	0	50	0	0	25	13	13	38

Table 3. **Percentage of pine seedlings 1 and 4 months old when inoculated that developed galls 9 months after inoculation with *Cronartium quercuum* f. sp. *fusiforme* or *C. quercuum* f. sp. *virginianae* from the United States**

F. sp. of inoculum and *Pinus* species	% seedlings that developed galls[a] 1-month-old	4-month-old
fusiforme		
taeda	74	36
elliottii	84	22
virginiana	1	0
densiflora	1	0
thunbergii	1	4
virginianae		
taeda	2	2
elliottii	11	1
virginiana	25	46
densiflora	5	4
thunbergii	28	15

[a] Average for five flats of 20 seedlings each.

Table 4. **Number of galls formed by *Cronartium quercuum* on various pine species in Japan[a]**

Pinus species	Country of seed origin	Number of trees	Number of galls per tree: Range	Mean
banksiana	USA	42	0	0.0
contorta	USA	4	0	0.0
pungens	USA	5	0	0.0
rigida	USA	48	0	0.0
virginiana	USA	8	0	0.0
tabulaeformis	China	8	0	0.0
ponderosa	USA	11	0-2	0.3
nigra var. *austrica*	Denmark	5	0-1	0.2
nigra var. *poiretiana*	Sweden	5	0-1	0.2
densiflora	Japan	7	0-1	0.1
thunbergii X *densiflora*	Japan	36	0-5	0.8
montana	Sweden	30	0-70	6.2
sylvestris	Sweden	49	0-38	6.9

[a] Twenty-seven-year-old trees at Tohoku Tree Breeding Institute, Iwate Prefecture, Japan.

infection on both loblolly and slash pine dropped dramatically. Inoculations of 1- or 4-month-old *P. taeda* and *P. elliottii* var. *elliottii* seedlings with *C. quercuum* f. sp. *virginianae* resulted in very little infection. However, there was more infection on the 4-month-old *P. virginiana* seedlings than on the 1-month-old seedlings. A very small percentage of *P. densiflora* seedlings developed galls at either age. In contrast, the 1-month-old *P. thunbergii* seedlings were as heavily infected as the normal host species, *P. virginiana*.

To date, the inoculations of American pines in Japan with the *P. densiflora* strain of *C. quercuum* have been negative. Dr. Kaneko assessed the susceptibility of several native and exotic pine species to the Japanese *C. quercuum* under field conditions. These 27-year-old trees were in research plots in the Iwata Prefecture in Japan (Table 4), and were probably exposed to spores originating from *P. densiflora*. There was no rust present on *P. banksiana*, *P. contorta*, *P. pungens*, *P. rigida*, and *P. virginiana* from the USA, or on *P. tabulaeformis* from China. *Pinus ponderosa* and the two strains of *P. nigra* had only a few galls. *Pinus densiflora* and the cross between *P. thunbergii* and *P. densiflora* also had relatively few galls. The species with the highest rate of infection were *P. montana* and *P. sylvestris* from Europe.

DISCUSSION

The inoculations of oaks in both North America and Asia indicate that *Q. rubra*, the northern red oak from North America, seems to be almost a universal host for the rust fungi within the pine-oak group, since there was at least some infection on this species in all inoculations. Secondly, most oak species from both continents were susceptible to infection by rust isolates from both continents. The oak inoculations in the USA showed some possible differences in virulence between *C. quercuum* f. sp. *fusiforme* and *C. quercuum* f. sp. *virginianae*, based on the infections on *Q. serrata*. However, only a few seedlings of *Q. serrata* were tested with each *forma specialis*, and further work is needed on this point. Although the inoculations of American pines in Japan have been negative, interesting results were obtained from the 27-year-old field plantings of pine in that country. The two European species in those plantings were the most susceptible hosts for the *densiflora* strain of the rust. This result suggests that this particular strain of pine-oak rust could be more damaging in Europe, where both *P. sylvestris* and *P. montana* are native, than it is in Japan and Korea, where *C. quercuum* is normally found.

Inoculations with *C. quercuum* f. sp. *fusiforme* in the United States have not produced disease symptoms on Japanese red and black pines. Infection after inoculations with f. sp. *virginianae*, however, indicates that *P. thunbergii* is almost as good a host for this rust as its native host, *P. virginiana*. This result raises some questions about the relationship between these two pine species that would cause them to be so similar in response to this pathogen. It also means that there should be more concern about introducing *C. quercuum* f. sp. *virginianae* into Japan and Korea than f. sp. *fusiforme*.

As is the case with many experiments, our investigations have raised more new questions than they have provided answers. However, we feel that the results from our three-country comparisons have been interesting and have provided some information that may lead to new research on the rather complex interrelationships between the various strains of *Cronartium quercuum* that make up the pine-oak complex.

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A STUDY OF XINGKAI LAKE PINE GALL RUST

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INTRODUCTION

The Xingkai Lake pine (*Pinus takahasii* Nakai) is geographically distributed in Mishan, Jidong, near Xingkai Lake in eastern Heilongjiang province. This pine is closely related to *P. sylvestris* but differs in minor morphological characteristics. The area is generally 400-1000 m above sea level and has lakeside dunes and mountain-ridge rocky soils. The Xingkai Lake pine is one of the most valuable softwood timber species, and the forest it forms not only has commercial value but serves as the lakeside shelterbelt and as a scenic attraction in the area.

The pine gall rust caused by *Cronartium quercuum* (Berk.) Miyabe ex Shirai is a serious stem and branch disease. The infection history of Xingkai Lake pine gall rust goes back over half a century. No research and investigation had been done until 1985-87, when the writers conducted intense and systematic research on the disease. The research was initiated with a germination test of the parasite and an artificial inoculation experiment. The pathological anatomy was studied to compare the infected and healthy tissues in the laboratory; and the natural regeneration, fruiting ability, and growth rate differences between infected and healthy forest stands were investigated in the field. Through research, we understand the biological characteristics of the parasite and the disease occurrence and its development characteristics, as well as the impact of the disease on the forest. These obviously provide the basis for implementing effective control measures.

MATERIALS AND METHODS

Disease Investigation

Investigation was implemented along Xingkai Lake by using the line survey method in 1- to 2-km sampling intervals. The incidence of disease was recorded in different intervals and a lateral branch or tree stem bearing galls representing each interval was collected for laboratory anatomical analysis. The infection grades classification is listed in Table 1.

Observation of Parasite

Seasonal development of the disease on pine and oak was observed in the field. All kinds of spores were collected from hosts in the field and examined and measured under light microscopy and electron microscopy.

Table 1. **Classification of infection grades of Xingkai Lake pine gall rust**

Grades	Represent values	Incidence levels	Classification standards
I	0	Healthy	No infection
II	1	Lightly infected	Only lateral branch infected with 1-5 galls for each branch, without withered branch caused by infection
III	2	Moderately infected	Lateral branch with 6-30 galls, main stem with or without galls
IV	3	Heavily infected	Lateral branch bearing more than 30 galls, main stem half encircled, more than half branch withered
V	4	Dead	Main stem encircled, all branches with galls, tree will die

Artificial Inoculation Experiment

In May and June, aeciospores and urediospores collected from infected pines and oaks were artificially inoculated onto healthy oak leaves, both inside the greenhouse and in the field. To maintain the desired humidity, plastic bags were used as covers for 48 h, then removed to allow natural growth. In the following days, observations were made and the time recorded when infection signs appeared. The controls were conducted at the same time.

In September, oak leaves collected from infected trees were soaked in water for a few minutes, then attached to Xingkai Lake pine and Mongolian pine needles and young stems. Plastic bags were also used to keep them damp for 48 h, then they were let grow naturally. The same procedure was used for five comparison trees. Late the following May, the inoculated seedlings were collected. Freehand sections were cut directly from young stems with necrotic areas and put into 0.1% acid fuchsin in saturated chloral hydrate, then observed under the microscope.

Biological Characteristics of the Parasite

Effect of Temperature on Spore Germination

Freshly collected aeciospores and urediospores were dispersed on 2% water agar by an atomizer, then treated under a temperature series of 4, 8, 12, 16, 20, 24, and 32°C with three repetitions for each temperature level. Observations were made in three microscope fields at 5-h intervals, and the number of spores germinated were recorded.

Twenty telial columns cut from freshly collected oak leaves were put onto 2% water agar glass slides. The temperature treatment and observation were the same as for aeciospores and urediospores.

Infected oak leaves were soaked in water for a few minutes, then taken out and put in plates to collect the basidiospores. These were treated at the different temperatures and observed by the method above.

Effect of Humidity on Spore Germination

Aeciospores and urediospores were dispersed onto both 2% water agar medium and clear glass slides which were put into different humidity environments at a temperature of 12°C. Examination was made 20 h later.

Telial columns were put on 2% water agar medium slides with 20 telial columns each, and 1-cm telial leaves were put on clear glass slides. Both were treated under 18°C temperature and controlled humidity for 24 h, then examination was made.

The 2% water agar and clear glass slides with basidiospores were treated under 20°C temperature and different humidity conditions and examined 36 hours later. The method of collecting basidiospores was the same as described earlier.

Effect of Light on Spore Germination

Freshly collected aeciospores, urediospores, telia, and basidiospores were each put on 2% water agar plates and held at 20°C. The light treatment was direct light (40-W incandescent lamp), dark, natural light-to-dark, or natural light. Three repetitions of the four light treatments were made to examine the germination of spores.

Anatomy of Infected and Healthy Tissues

In October 1985, infected and healthy tissues from 5-year-old infected trees were cut and the specimens were softened in glycerin alcohol for about a week. Sections 15 µm thick were cut by sliding microtome. Other specimens were first fixed in FAA solution embedded in paraffin, then sections 10 µm thick were cut on a rotary microtome. Safranin-fast green and hematoxylin stains were used to differentiate various tissues and mycelium in the gall tissues. Infected and normal wood was cut into small pieces, then boiled and macerated in 40% $HN0_3$ solution. All sections were mounted in Canadian balsam.

Spread of Aeciospores

In mid-May 1985, in heavily infected forest stands, spore traps were placed 1, 3, 5, 14, 30, 50, and 100 m horizontally from infected trees, and 1, 3, and 5 m vertically at ground level. Each slide glass was fixed in four directions of the traps, and examined at 8:00 a.m. and 2:00 p.m. The corresponding weather reading of air temperature and humidity, and wind speed and direction were recorded.

Control Experiment

Twelve fungicides were mixed with diesel and soap solution in 1:100 and 1:300 volume ratios. These mixtures were sprayed onto 2% water agar plates and then covered with an aeciospore layer, by atomizer. All treatments were replicated three times, and each plate was examined 48 h later at room temperature and at three magnifications.

In May 1985, infected trees with galls but without spores were selected and tagged. About eight galls were coated with each fungicide and checked in June and the following January.

In October 1986 and May 1987, the mixed fungicide of pine tar and diesel oil (1:5 in volume) was used for the control experiment; 523 galls from 199 trees and 744 galls from 206 trees were coated with the mixture. These were checked randomly in May and June of 1987.

Effect of Gall Disease on Forest and Trees

Seeding and Seed Quality

Three sample trees were selected from each of healthy and infected forest stands with the same site conditions, with respective ages of 23 and 17 years. Cones of each sample tree were counted, then the mean cone number of each selected tree was calculated. Two hundred mature cones from each of the healthy and infected trees were collected, of which 100 were used for measuring the cone properties. Seeds collected from these randomly sampled cones were further examined by X-ray to obtain the seed quality information.

Natural Regeneration

Natural regeneration was investigated for differences between healthy stands (infection grade I), lightly infected stands (infection grade II), and moderately infected stands (infection grade III). The sampling method was also used for 40-m^2 site units randomly selected and representing each infection grade. All seedlings and saplings below 1.3 m in height, as well as the number infected by gall rust, were recorded in each subsample unit.

RESULTS

Results of Disease Investigation

The disease investigation has shown that pines at the edges of stands were not infected; however, the inner part of stands was seriously infected. The incidence of disease in heavily infected stands can reach 98%, and the infection increases with time (Table 2).

Table 2. Disease investigation (1985)

Sample no.	Infection				Incidence of disease (%)	Index of disease	Remarks
	Time	Part	Age	History			
1	-	-	-	-	-	-	A few telial columns on oaks
2	-	-	-	-	-	-	As above
3	1959	Branch	3	26	5.26	1.32	
4	1924	Branch	4	61	98.4	35.15	
5	1939	Branch	3	46	96.8	41.66	
6	1942	Branch	5	43	94.2	45.93	
7	-	-	-	-	-	-	No pine, a few telial columns on oaks
8	1957	Branch	6	28	4.08	2.04	
9	1946	Branch	3	39	95.7	45.65	
10	1950	Branch	5	35	13.6	10.45	
11	1964	Branch	7	21	84.3	33.57	
12	1965	Stem	3	23	79.1	30.52	
13	1952	Branch	3	33	10.3	2.56	
14	1971	Branch	3	14	20.6	6.74	
15	-	-	-	-	-	-	No telial columns on oaks

Observation of Parasite Morphology

The spermogonium of *Cronartium quercuum* forms in the cortex of Xingkai Lake pine in October. The orange spermatial drops with abundant spermatia exude from rupturing gall bark in January. Spermatiophores are 1.8 μm in width and 19.8 μm in length (Fig. 1). The spermatia are 4.5-5.4 × 1.8-2.7 μm (Fig. 2). Aecia form in mid-May; aeciospores are orange, in chains, oval or elliptical, and 29-38 × 21-28 μm. The surface morphology of aeciospores is the same as described by Hiratsuka (1971) and Tong (1979) (Fig. 3, 4). In early June, uredia form in oak leaves, the alternate host. Urediospores are orange, oval or subglobose, and 19-30 × 13-23 μm (Fig. 5). In late June, the dark telial columns form from uredia; the teliospores are oblong and 49-35 × 41-23 μm. From mid-August to October, teliospores germinate to produce hyaline basidiospores that measure 11.3-15.5 μm.

Artificial Inoculation Experiments

The results of inoculation with aeciospores and urediospores showed that old leaves were resistant to infection but that younger leaves were susceptible. Even though some old leaves were infected, they did not form uredia.

Pine seedlings inoculated with basidiospores developed brown necrotic areas and ruptured cortex.

1
2
3
4
5
00067 HU2 0.5U
00068 HU2 0.5U

Figures 1-5. **1.** Cross section of *Pinus takahasii* stem with a spermogonium of *Cronartium quercuum* (× 450). **2.** Spermatia (× 528). **3.** Aeciospore of *Cronartium quercuum* (× 20000 SEM). **4.** Aeciospore of *Cronartium quercuum* (× 8000 SEM). **5.** Urediospores of *Cronartium quercuum* (× 3000 SEM).

Biological Characteristics of Parasite

Effect of Temperature on Spore Germination

The germinative characteristics of aeciospores and herediospores are similar in regard to temperature, both starting germination at an optimum of 12°C and over a range of 4-32°C (Fig. 6, 7). Under suitable temperature, aeciospores and urediospores germinate in 3 h, reaching the germination peak in 15 h. Twenty hours later, most germ tubes undergo lysis at the distal end. Most germ tubes are unbranched and a few are spirally branched. Germ tubes of spores are short, thick, and twisted at 28-32°C. At 4°C, the germ tube can develop normally, but germination is slow.

The teliospores germinate at 8-28°C, but the optimum temperature is 16-18°C. The telial columns placed in each of 32°C and 4°C environments for 24 h were moved to favorable conditions. The former did not produce basidiospores; but the latter germinated normally and produced a great number of basidiospores (Fig. 8).

Basidiospores that telia produced at 8°C did not have the ability to eject forcibly when the basidiospores were moved to 10, 14, and 28°C. Only a few can shoot off but do not germinate in 48 h. In the basidiospores produced at 18°C and moved into the three temperature environments, germination rate was very high.

Basidiospores germinated at temperatures from 8 to 28°C, but the optimum temperature range was 18-20°C (Fig. 9). Basidiospore color darkened before germination, and secondary basidiospores were formed. Each of these produced a branched germ tube.

Effect of Humidity on Spore Germination

Germination tests show that all types of spores of *Cronartium quercuum* are more affected by free water on the substrate surface than by relative humidity.

Effect of Light on Spore Germination

All types of spores germinate best under natural conditions of light to dark. Direct light will inhibit germination even if followed by darkness (Fig. 10).

Anatomy of Infected and Uninfected Tissues

Examination of the anatomy of *Cronartium quercuum* galls shows that rust hyphae freely ramify through the intercellular spaces within parenchymatous tissues of the cortex, phloem, cambium, and xylem. Hyphae are perennial and large (diameter varying from 1.8 to 5.4 µm, averaging 3.6 µm), and most hyphae are in the cortex. Haustoria are cylindrical with rounded or blunt ends (3.6 µm in width, 5.4-23.4 µm in length) that penetrate cells through the parenchyma cell wall (Fig. 11) and can invade tracheids in the xylem (Fig. 12). Parenchyma in gall tissues is observed to be hypertrophic or hyperplastic. Vertical tracheids in gall xylem are short and abnormal in shape (Fig. 13, 14, 15, 16, 17, 18).

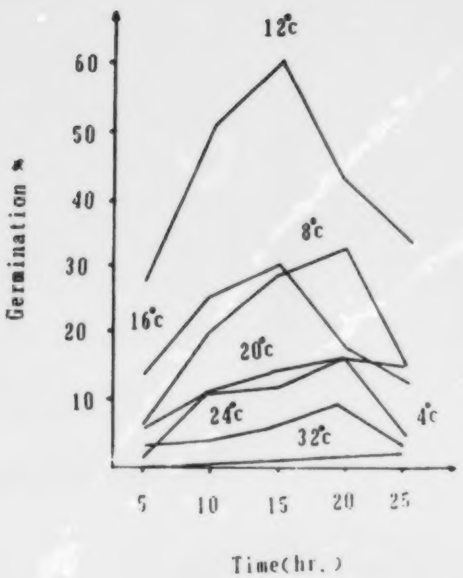


Figure 6. **Effect of temperature on germination of aeciospores at various times.**

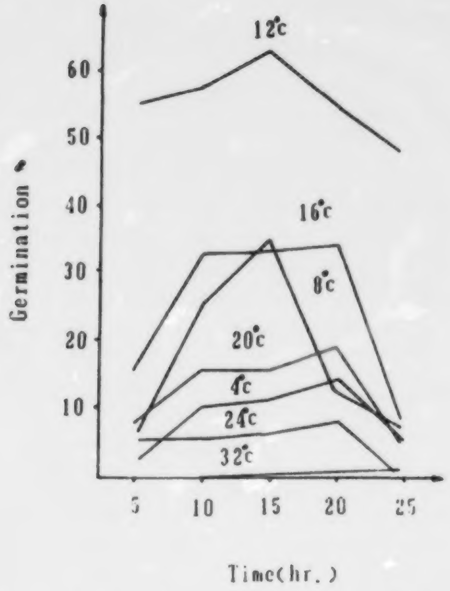


Figure 7. **Effect of temperature on urediospores at various times.**

Time (hr.)	8	10	12	14	16	18	20	24
13	C	D	E	E	E	E	E	D
35	B	D	D	E	E	E	E	D
30	B	C	D	E	E	E	E	D
25	B	B	D	D	E	E	E	D
20	B	B	C	C	E	E	D	D
15	A	B	B	B	D	D	D	D
10	A	A	A	A	C	C	C	C
5	O	O	O	O	B	B	B	B
3	O	O	O	O	A	A	A	O

Temperature °c

Figure 8. **Effect of temperature on germination of telial columns at various times.** Six levels of germination were recognized: O = no germination; A = producing basidiola; B = producing basidia in 1/4 of telial column; C = producing basidia in 1/2 of telial column; D = producing basidia in 3/4 of telial column; E = producing basidia in whole telial column.

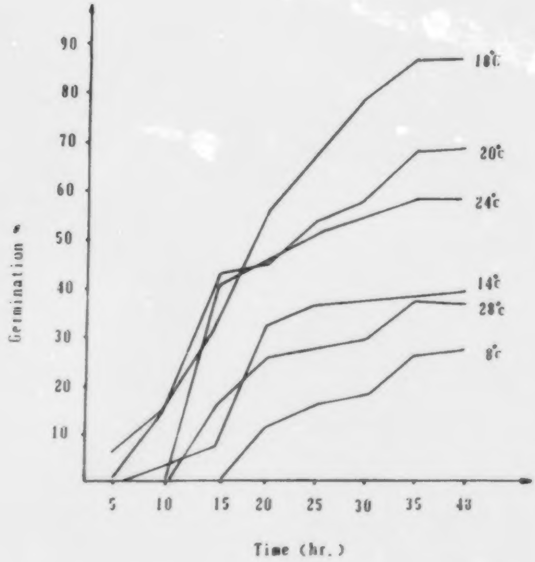


Figure 9. **Effect of temperature on germination of basidiospores at various times.**

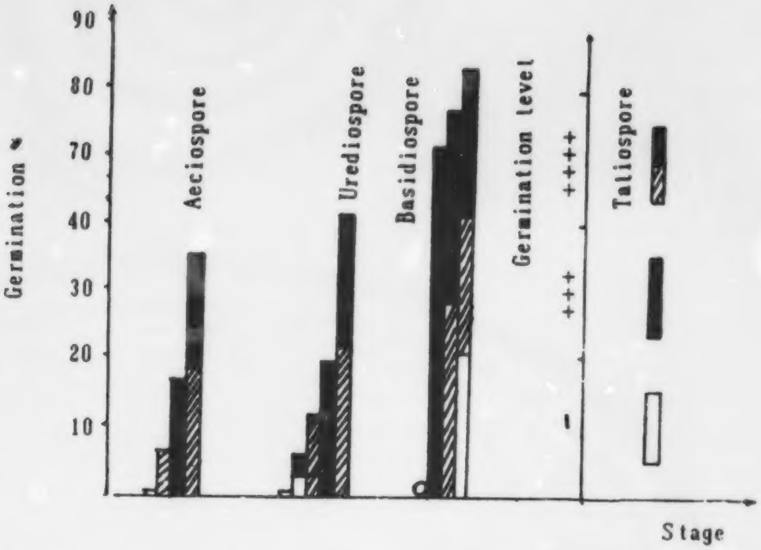


Figure 10. Effect of light on germination of various stages of spores.
++++ = 4/4-3/4 telial columns germination
+++ = 1/2-3/4 telial columns germination
☐ Light ▨ Natural light
■ Dark ◧ Light/Dark
◧ Light/Natural light/Dark
o = No germination/Light

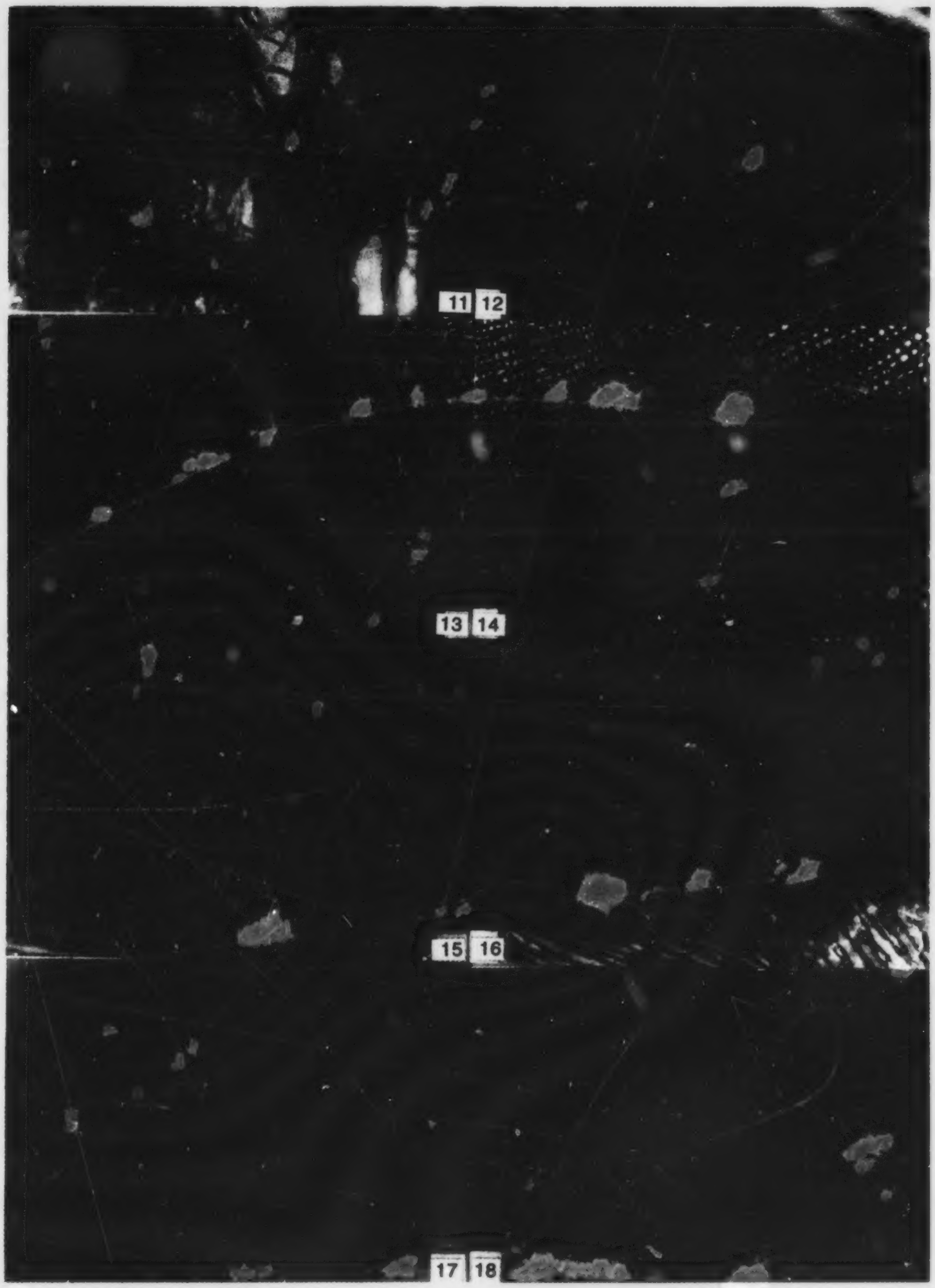
Details of the anatomy of gall and healthy tissues (Table 3) are similar to some reported by Jackson et al. (1958) and Jewell et al. (1962, 1965, 1967, 1975).

Aeciospore Release

From the field experiment, we found that 1) aeciospores dispersed mostly southward (62% of spores dispersed in this direction); 2) aeciospore dispersal increased dramatically 2-3 days after rain; 3) dispersal in the morning was much greater than in the afternoon, amounting to 56% of the whole day; 4) aeciospores dispersed mostly within 15 m from the infected trees, but some were found 100 m from the infected trees; 5) aeciospores mostly dispersed vertically in the area 3 m from the tree; and 6) aeciospores dispersed mostly in the period between May 23 and June 3.

Control Experiment

Results of the laboratory control experiment showed that all fungicides selected had 99.6% inhibiting efficiency to aeciospore germination. In the field coating experiment, the coating agents of pine tar and pine tar with diesel oil (1:1, 1:3, 1:5) gave satisfactory results and better infiltration and poisoning ability.



Figures 11-18. **11.** Intercellular hyphae and haustoria in cortex (× 528). **12.** Haustoria in wood rays (× 528). **13.** Transverse view of normal xylem (× 100). **14.** Transverse view of gall xylem (× 100). **15.** Tangential view of normal xylem rays (× 100). **16.** Tangential view of gall xylem rays (× 100). **17.** End of normal tracheid (× 400). **18.** End of gall tracheid (× 400).

Table 3. Average values (μm) for six anatomical characteristics in 10 samples of rust-infected and normal xylem of Xingkai Lake pine

	Tracheid		Ray-cell			Diameter
Characteristic[a]	Length	Width	Height	Width	Length	of resin
Gall tissue	694.3	28.4	25.0	22.14	76.8	71.5
Normal tissue	971.5	15.5	24.9	13.3	51.4	33.5
Calculated values	4.86	9.5	0.04	4.65	3.83	4.73
Significant difference 1%	**	**	NS	**	**	**

[a] Values represent the average of 50 sample measurements.

In October 1986 and May 1987, a large-area control experiment was conducted. When 100 random galls from different parts of the experiment site were checked, spermogonia and aecia were not found. Urediospores and telia produced on oak leaves were rare in the control area, but oak leaves in the compared blocks were densely covered with telial columns.

Effects of the Disease on the Forest

Relationship Between Infection Grade and Seeding Ability

Pine gall rust has a serious effect on the seeding ability of Xingkai Lake pine. The younger the tree, the more seriously it will suffer from the disease. The statistical *t*-test also showed that the cone lengths from healthy trees and infected trees (grade III) were distinctly different. The seed characteristics are also different in that seeds from healthy trees are plump with long wings, but those from infected trees are shriveled with short wings.

The X-ray examination indicated that the embryo and endosperm of seeds from healthy trees were well developed and had strong germination potential, but it was difficult to find well-developed seeds from infected trees (Fig. 19, 20).

Relationship Between Infection Grade and Natural Regeneration

Significant differences in natural regeneration frequency existed between different infection grades, ranging from 45% for healthy stands to 33% for lightly infected stands and to 24% for moderately infected stands in which 70% of seedlings were infected.

Relation Between Infection Grade and Annual Mean Increment in Diameter, Height, Volume

Analysis of variance of the annual mean increments in diameter, height, and volume between healthy trees and grades II and III infected trees showed significant differences among them. Further multiple Q-tests showed significance in the following paired infection grades: healthy and grade III, and

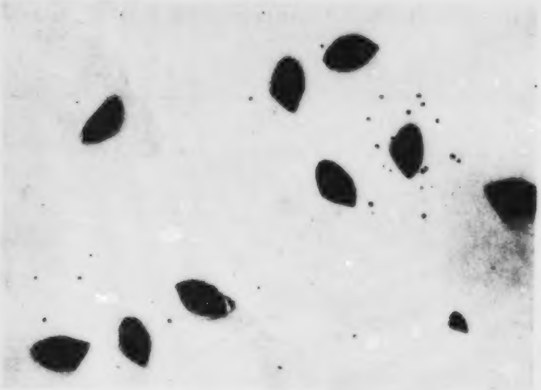


Figure 19. Normal seeds.

Figure 20. Abnormal seeds.

grade II and grade III in diameter mean increment; healthy and grade III in mean height increment; and healthy and grade III in mean annual volume increment.

Relationship Between Infection Grade and Losses of Diameter, Height, and Volume

As trees become infected to grade III level the annual mean increments in diameter, and height are reduced respectively by 57.4 and 80.5% from healthy trees.

DISCUSSION

The germination and ecological characteristics of various spores from this study indicate that the morphological characteristics are the result of lengthy adaptation to the environment. Aeciospores and urediospores, by growing in chains and heaps, favor dissemination. To acquire enough sunlight and free water for their maturation and germination, most spores develop surface ornamentations to increase their surface. This characteristic also increases their ability to adhere and to withstand extreme environmental conditions. Telia connecting to columns take advantage of breaking the thick epidermis of mature leaves to ensure every spore has the chance of accepting enough sunlight and free water to eject from the leaves. Formation of secondary basidiospores is an example of basidiospores adapting to the natural environment. The experiment has shown that basidiospores can form secondary basidiospores and germinate in 3 h at temperatures of 18-20°C. The formation period of secondary basidiospores can expand to several dozen hours if conditions are not suitable.

From experiments, we find that the temperature for telial germination is a vital factor in basidiospores germinating normally. In the study area, the mean temperature in August is 22.4°C with a low of 20.5°C at night, and the temperature in September is 14.1°C with a low of 10.9°C at night. This temperature variance suggests that large-scale germination of teliospores and infection of the host occur mostly in mid- and late-August.

That all spores could not germinate normally when exposed to direct light may be explained by the fact that urediospores and teliospores grow on the underside of the leaves to adapt to the environment. Although some urediospores were found on oak leaves directly exposed to sunlight, few of them developed into teliospores. In fact, most of the severely infected trees are located on level to gently sloping land with densely distributed pine trees and short oak trees. In contrast, the lightly infected areas are located on hillocks with high oaks exposed to direct sunlight.

Aeciospores play an important role in long-distance spreading and primary infection in new areas. This is due, first, to their having longer life than urediospores and to their greater abundance and, secondly, to their becoming mature just at the leafing season of oak. The infection area will be quite large if a suitable environment is provided. That telia have been found on oak leaves 1 km from severely infected areas is obviously the result of infection from aeciospores.

Considering that all leaves have the same chance to be infected by aeciospores and urediospores, and that old leaves were less infected than new leaves, we must think that stomata are not the only path for spore invasion. The epidermis must also be taken into account.

Although old leaves can be infected, the disease will not go through the uredial stage but instead go directly to the telial stage. The reason could be that the old leaves have a thick, horny layer that prevents urediospores from breaking out or that some urediospore-promoting materials inside the host decrease when leaves grow older, so that the formation of urediospores is limited.

Inoculation of pine with basidiospores has shown that invasion starts in the young stem. It takes only a half-year for a brown necrotic area to form in the cortex. Quite a large number of spores formed by schizohyphae have been found in necrotic areas. This phenomenon has also been found in naturally infected seedlings, but it is not clearly known what role it plays in infection.

Miller and Matthews (1984) reported a successful example of inoculation from wounds. Our investigation also found a 10-m-high tree having no galls on the stem but having five galls on roots exposed above the soil surface. This supports the probability of infections from both artificial wounds and natural wounds. If a woody gall forms, it expands 0.3-1.0 cm per year horizontally and 0.1-0.4 cm per year vertically.

The authors also noticed that galls from Xingkai Lake pine and from Scots pine (*P. sylvestris* var. *mongolica*) are different in form. Whether this difference is related to distribution of pathogen inside the host, its selection of host tissues, depth of invasion, and growth speed needs to be studied further to decide if *forma specialis* exists in China.

The Xingkai Lake gall rust has now extensively threatened the Xingkai Lake pine forest. When the infection reaches grade III, heavy losses will occur in the increments of diameter, height, and volume. We suggest that infection should be controlled to not exceed infection grade III by using pine tar plus diesel oil coating on galls every spring and autumn.

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RUSTS OF PINE FROM INDIA

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In a tropical country like India, *Pinus* is found growing in the hills with subtropical or temperate climates. In addition, some pines are grown as ornamentals at lower altitudes.

Six species of *Pinus* are reported from India. Four are distributed in the Himalayas: *Pinus insularis* Endl. (= *P. khasya*), *Pinus gerardiana* Wall ex Lamb., *Pinus roxburghii* Sarg. (= *P. longifolia* Roxb.), and *Pinus wallichiana* A.B. Jacks (= *P. griffithii*). Of the remaining two, *Pinus armandii* Franch. is known to occur in the North East Frontier Agency and *Pinus merkusii* Jungh & de Vriese grows in Burma and extends into eastern parts of the Himalayas.

Apart from the indigenous species, some exotic species also occur: *Pinus canariensis* C. Smith, *P. caribaea* Morelet, *P. cembroidevees* Zucc. var. *calabrica*, *P. patula* Schi. & Cham, *P. pinaster* Ait., *P. radiata* D. Don, *P. sabiniana* Doug., *P. taeda* L., and *P. thunbergii* Parl.

Major contributions towards the study of rust of *Pinus* from India have been made by several workers, such as Barclay (1887, 1890a, b), Butler (1905), Khan (1928a, b), Arthur and Cummins (1933), Sydow and Butler (1906, 1912), Bagchee (1929a, b, 1933, 1940, 1950a, b, c), Puri (1955), Pandotra and Ganguli (1964), Bakshi (1964), Bakshi and Singh (1972), and Bakshi et al. (1972).

Butler and Bisby (1960), Bilgrami and Jamaluddin (1979), and Mukerji and Bhasin (1986) have recorded the occurrence of *Coleosporium barclayense* Bagchee, *C. companulae* Lev. ex Kichx., *Cronartium himalayense* Bagchee, *C. quercum* (Berk.) Miyabe ex Shirai, *C. ribicola* J.C. Fischer, and *Melampsora oblonga* Bagchee on the host genus *Pinus*. In the present paper a revisionary study of the pine rusts from India are made. The host range and geographical distribution of individual species are also given.

***Coleosporium barclayense* Bagchee**. Indian For. Rec. N.S. Bot. 4:53. 1950.
Peridermium brevius (Barcl.) Sacc. (1981) Syll. Fung. 9:327.

Literature: Barclay, A. (1890). Asiat Soc. Bengal 59:6-9.
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Bagchee, K.D. (1950b) Indian For. Rec. N.S. Bot 4:43-61.
Puri (1955) For. Bull. (N.S. Mycol.) 179:1-10.

Hosts: O & I on *Pinus wallichiana* A.B. Jacks. (= *Pinus excelsa* Wall.) II & III on *Senecio elatus* and *S. rufinervis*.

Distribution: Dharmasala, Kulu, Simla, (H.P.), Mussorie, Nainital, Chakrata (U.P.), Kashmir (J. & K.)

Exsiccati: IMI 66229, IMI 203354, IMI 76034, IMI 301031.

Remarks: Bagchee conducted a series of experiments in 1934, 1935, and 1936 making cross inoculations on *Pinus wallichiana* and *Senecio rufinervis*. During these experiments he found a new species of *Coleosporium* and named it *Coleosporium barclayense*. As per Bagchee (1950), *C. barclayense* differs from *C. senecionis* in having harder urediniospores and teliospores and is much more serious by attacking all the needles in a fascicle with 3-30 aecia per needle.

***Coleosporium companulae* Lev. ex Kickx.** Fl. Flanders 2:54. 1967.
Peridermium orientale Cooke (1877), Indian For. 3:91.

Literature:
Barclay, A. (1980b) J. Asiat. Soc. Bengal 59:75-112.
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Anonymous (1950) Indian J. Agric. Sci. 20:107-142.
Pandotra, V.R., and Ganguly, D. (1964) Mycopathol. Mycol. Appl. 22:106-116.

Host: O & I on *Pinus roxburghii* Sarg. (= *Pinus longifolia* Roxb.) and *P. wallichiana* Jacks. II & III on *Cephalostigma schimperi, Companula colorata* and *C. canescens.*

Distribution: Naterhat (Bihar), Jammu (J. & K.), Dharmasala, Simla (H.P.), Almora, Dehra Dun, Nainital (U.P.), Darjeeling (W.B.).

Exsiccati: IMI 60955, IMI 66230, IMI 76035, IMI 156515, IMI 95538, IMI 239381, IMI 259251.

Remarks: Bagchee (1940) mentions that the natural infection of *Pinus roxburghii* (*P. longifolia*) needles takes place from September to November. If weather is warm the spermagonia appear by October, and a few aecial sori may even appear in November. The main crop of aecial sori appears later in February until March. As proved by the inoculation experiments, the infection of pines takes place from the telial stage on *Companula colorata.* Teliospores on germination cause the infection on pine needles between the months of September and November. The bursting of aecial sori and dissemination of aeciospores takes place from March to May.

***Cronartium himalayense* Bagchee.** Nature 124:691-692. 1929b.

Literature:
Bagchee, K.D. (1929a) Indian For. Rec. (Bot.) 14:1-24.
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Bagchee, K.D. (1940) Indian For. Rec. (N.S. Bot.) 1:247-266.
Bagchee, K.D. (1944) Indian For. 70:323-325.
Bakshi, B.K., and Singh, S. (1972) Indian For. 98:239-240.

Hosts: O & I on *Pinus roxburghii* Sarg. (= *P. longifolia* Roxb.) and also on *P. canariensis* C. Smith. II & III on *Swertia alata, S. angustifolia, S. chirete* and *S. cordata.*

Distribution: Almora, Chakrata, Garhwal, Mussorie, Nainital, Ranikhet (U.P.), Kulu, Simla (H.P.), Naterhat (Bihar).

Remarks: *Cronartium himalayense* is the most common and destructive of all pine rusts from India. Young saplings and poles are severely attacked and killed outright. Aecia appear during April-July. In most cases the death of defected trees takes place during the season of production of aeciospores, and in a few cases it takes place after the appearance of the second crop of aecial sori. The susceptible age of the plant is from seedling to sapling stage (20 years). Older trees are seldom invaded. Affected parts show spindle-shaped swellings confined to bark.

***Cronartium quercum* (Berk.) Miyabe ex Shirai.** Botan. Mag. Tokyo 13:74. 1899.

Literature: Anonymous, Indian J. Agric. Sci. (1950) 20:107-142. Bagchee, K.D. (1950c) Indian For. 76:216-220.

Hosts: O & I on *Pinus insularis* Endn. (= *P. khasya* Royle.) II & III on *Quercus prinoides* Willd.

Distribution: Shillong (Assam).

***Cronartium ribicola* Fisher. ex Rabh.** Fg. Eur. No. 1595. Hedwigia 11:182. 1872.

Literature: Nisbet, J. (1985) Indian For. 21:126-133.
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Bagchee, K.D. (1950a) Indian For. Rec. (N.S. Bot.) 4:1-41.
Puri, Y.N. (1955) For. Bull. (N.S. Mycol.) 179:1-10.
Bakshi, B.K., Reddy, M.A.R., Puri, Y.N., and Singh, S. (1972) P.L. 480 Rep.

Hosts: O & I on *Pinus wallichiana* A.B. Jacks (= *P. excelsa* Wall.). II & III on *Ribes* sp.

Distribution: Dehra Dun (U.P.), Kulu, Simla (H.P.), Kashmir (J. & K.), Bashahr (Punjab).

***Melampsora oblonga* Bagchee**, Indian For. Rec. (N.S. Bot.) 4:57. 1950.

Literature: Puri, Y.N. (1950) For. Bull. (N.S. Mycol.) 179:1-10.

Host: On *Pinus wallichiana*, A.B. Jacks (= *P. excelsa* Wall.).

Exsiccati: Central Himalaya, Chakrata (U.P.), India (Type).

Remarks: *Melampsora oblonga* Bagchee is the second *Melampsora* species on Pinaceae showing microcyclic life cycle. This species, like that of *M. farlowii*, is a "lepto-form". Bagchee (1950) through his inoculation experiments confirmed the microcyclic nature of this rust fungus.

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CONTROL OF FUSIFORM RUST ON LOBLOLLY AND SLASH PINE SEEDLINGS IN FOREST NURSERIES IN THE SOUTHEASTERN UNITED STATES

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Fusiform rust, caused by *Cronartium quercuum* (Berk.) Miyabi ex Shirai f. sp. *fusiforme* Burdsall and Snow, is one of the most important diseases of loblolly (*Pinus taeda* L.) and slash (*P. elliottii* Engelm. var. *elliottii*) pine seedlings in forest nurseries in the southeastern United States. An effective rust control program is essential in ensuring that the almost two billion pine seedlings produced annually in the southeastern United States leave the nursery rust-free. Since the early 1980s, the systemic fungicide triadimefon (Bayleton 50 WP) has been the fungicide of choice. This paper describes the fusiform rust infection period, the schedule of events in forest nursery management relative to the rust infection period, and the evolution of a fusiform rust control program using Bayleton 50 WP.

FUSIFORM RUST INFECTION PERIOD

The life cycle of *C. quercuum* f. sp. *fusiforme* is well documented and has been critically reviewed (Czabator 1971). However, for the purpose of detailing the infection chronology in nursery beds, a brief review is necessary. The aecial stage occurs on infected pines in March. Wind-disseminated aeciospores infect newly developing oak leaves, where the uredial and telial stages occur in rapid succession. By mid- to late April mature telial horns have formed. The cells comprising the telial horns germinate in place, and release basidiospores (sporidia) that are airborne and serve as sources of infection to pines. Depending on the temperature and humidity, basidiospores may be released from April through early July, thus the infection periods for both oak and pine hosts are relatively well-defined.

SCHEDULE OF EVENTS IN FOREST NURSERIES

Both slash and loblolly pine seedlings are utilized in the southeastern United States as 1-0 stock. Seeds are sown on prepared nursery beds in April, and the seedlings are lifted for outplanting the following December through February. Seeds begin to germinate approximately 4 (slash) to 7 (loblolly) days after sowing; and susceptibility to fusiform rust infection commences at the time the radicle begins emerging, and continues throughout the life of the tree. Galls are not visible on infected seedlings until approximately seven months following infection. Few galled seedlings survive beyond a few months after outplanting, and those that do survive never develop into a marketable tree. Therefore, nurseries with a significant rust problem are obliged to inspect their seedlings, and cull those that are rust-infected, before packaging their product for shipment to the field.

EVOLUTION OF A FUSIFORM RUST CONTROL PROGRAM

Beginning in the early 1940s (Sleeth 1943), control of fusiform rust in forest nurseries was accomplished with either Bordeaux mixture or the fungicide Fermate (ferric dimethyldithiocarbamate).

Except for refinements (Siggers 1951) in the spray program, control of fusiform rust in forest nurseries remained unchanged for nearly 40 years. Nursery beds were sprayed a minimum of two times per week from seed-crack through early July; in cases of rainy weather, Fermate was applied three or more times a week. Frequent applications were necessary because Fermate is a nonmobile contact fungicide, and newly formed tissues had to be sprayed to be protected. Because of frequent passes of spray equipment across the nursery beds, losses of seedlings in the outer drill rows were commonplace. In addition, Fermate has no systemic activity, and rust infections that became established during the intervals between sprays proceeded to develop unhindered throughout the remainder of the growing season.

Several researchers (Sleeth 1943; Hare 1973; Hare and Snow 1976; Kelley 1978) had tested various systemic fungicides for efficacy against fusiform rust; but it wasn't until 1979 (Snow et al. 1979) that publication of test results with the fungicide triadimefon indicated that a new era of rust control in forest nurseries was on the horizon. By 1982, an effective rust control program had been worked out and petitions for Special Local Need labels (FIFRA 24-C, state labels) had been sent to most southeastern states. Soon after issuance of the 24-C labels, an EPA label was forthcoming that included a seed-soak procedure developed by Mexal and Snow (1978). In 1986, a seed-dressing procedure developed by Kelley (1985a) as an alternative to the seed-soak was included on the EPA label.

Since 1985, the recommended rust control program for forest nurseries in the southeastern United States has remained unchanged (Kelley 1985b). The program involves two steps, as follows:

1. Seed dressing with Bayleton 50 WP: Thoroughly mix triadimefon at a rate of 2.5 gm of Bayleton 50 WP/kg of seeds for 10 minutes in a tumbler apparatus. Seed surfaces should be wetted with water prior to mixing. Bird or animal repellent, such as Gustafson 42-S, may be applied following application of the triadimefon. After treatments, spread seeds to air dry before sowing. Seedlings from triadimefon-treated seeds are protected from rust for 26+ days after sowing.

2. Foliar sprays with Bayleton 50 WP: Apply foliar sprays at a rate of 0.56 kg of Bayleton 50 WP/ha per application. Apply through hollow cone nozzles at a spray volume of 325 L/ha or greater. Each hectare volume of spray should contain 0.47 L of the oil-surfactant blend Agridex. The first spray should be applied approximately 26 days after sowing and the final spray should be around June 15. If there are more than 3 weeks between the first spray and June 15, a spray should be applied midway between the first and last sprays. Properly applied foliar sprays provide protection for 21 days after application; they also provide kickback activity against rust infections that occur up to 7 days before application.

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MORTALITY IN RUST-INFECTED PLANTATIONS OF *PINUS ELLIOTTII* VAR. *ELLIOTTII* AND *PINUS TAEDA*: A 5-YEAR COMPARISON OF THINNED AND NONTHINNED AREAS

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Pine plantations occupy about 10 million hectares in the southern United States, and recent assessments indicate this area will more than double by the year 2030 (USDA Forest Service 1988). Slash pine (*Pinus elliottii* Engelm. var. *elliottii*) and loblolly pine (*Pinus taeda* L.) are the species most commonly planted in the south. They are also the species most susceptible to damage caused by fusiform rust (*Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*). Losses to rust result from mortality, stem defect, and a reduction in the growth of trees with severe stem infections.

More than half of the slash and loblolly pine plantations are greater than 10 years of age (Sheffield and Knight 1982; Sheffield et al. 1983), and many are heavily infected with fusiform rust. The selective thinning of trees with severe stem infections has been proposed as a means of reducing losses to rust in plantations where trees are large enough for commercial thinning (Powers et al. 1974; Belcher et al. 1977). A large-scale study is being conducted in Alabama, Georgia, and South Carolina to determine the effectiveness of salvage-sanitation cutting in merchantable slash and loblolly pine plantations (Belanger et al. 1985). Silvicultural and economic evaluations will be based on stand growth, total yield, and product value. Results and management recommendations will be strongly influenced by the amount and causes of mortality that occur in the stands.

This report quantifies and characterizes total mortality and rust-associated mortality (RAM) in thinned and nonthinned portions of the study plantations, for the first 5 years after treatment.

MATERIALS AND METHODS

Study Plantations

Ten slash pine plantations are located in the Coastal Plain of Georgia and South Carolina. Plantations were selected to include a wide range of stand and rust conditions. When they were thinned, the study plantations ranged from 13 to 23 years, and averaged 16 years, of age. Average diameter at breast height (dbh) was 15.7 cm. Incidence of trees with fusiform rust stem infections ranged from 35 to 70%.

Eleven loblolly pine plantations were selected in the Coastal Plain of Alabama and Georgia. When thinned, the loblolly plantations ranged from 15 to 22 years, and averaged 17 years, of age. Average dbh was 17.2 cm. Stem gall incidence ranged from 46 to 74%.

The areas of the plantations ranged from 20 to 80 ha. Approximately one-half of each plantation was designated for selective thinning and the remaining half was not thinned, as a basis for comparison. Four 0.10-ha permanent plots were established in the thinned and nonthinned areas of each plantation to assess the advantages and disadvantages of removing trees with severe stem infections. All trees were numbered to maintain their identities throughout the study. Individual tree measurements included dbh, number of stem and branch galls, crown class, and a risk class based on rust severity and the associated likelihood of mortality before rotation (22 to 30 years). Tree height and detailed rust characteristics were measured on a 20% subsample of the study population.

Guidelines for Tree Removal

The success of selective thinning in rust-infected plantations is based on anticipating mortality. The following risk criteria, based on rust severity, were the primary considerations used in the marking and removal of trees with stem infections:

High risk:	trees considered likely to die before rotation; stem girdling >50%, stem cankering >30%
Moderate risk:	tree survival to rotation age questionable; stem girdling 40-50%, stem cankering 20-30%
Low risk.:	trees likely to survive to rotation; stem girdling <40%, stem cankering <20%

Most high-risk trees were marked for removal; low-risk trees were not marked. Moderate-risk trees provided flexibility in the selection process; removals were based on potential value of trees, an assessment of production requirements, and spacing. Trees were cut and removed during all seasons of the year. Scheduling was strongly dependent on the availability of logging crews, weather, and local demands for pulpwood. Trees were removed from all risk classes to provide access for logging equipment.

Assessing Mortality

Study plots were surveyed annually to determine amount and cause of mortality. Rust-associated mortality was defined as any dead tree with a stem gall. Additional mortality was attributed to weather (lightning, wind, ice), insects, logging, suppression, other (fire, cutting for access rows), and a category classified as unknown.

Probability estimates for mortality during the 5-year study period were defined as

$$P = DT_2 : LT_1$$

where P = probability estimate, DT_2 = dead trees with stem galls at end of study period, and LT_1 = living trees with stem galls at beginning of study period.

RESULTS AND DISCUSSION

Slash Pine

The selective thinning of rust-infected stems in slash pine plantations removed an average of 348 trees per hectare (Table 1). Thinning levels of individual plantations were classified as moderate to heavy. Eighty-five percent of the trees cut were from the high- and moderate-risk classes. Basal area was reduced from an average of 22 m^2/ha to 15 m^2/ha. Thinning did not significantly change the average diameter or tree height of the plantations. Residual stocking in the thinned study plots averaged 732 trees per hectare compared to 998 trees per hectare in nonthinned plots. Treatment effects on rust characteristics are summarized in the paper by Miller et al. (these proceedings).

Total mortality in the nonthinned portions of the slash pine plantations averaged 15.63 m^3/ha during the 5 years since the study was initiated (Table 2). RAM accounted for 79% of the total volume loss. Most of the remaining mortality resulted from insect attacks and unknown causes.

Total mortality in the thinned portions of the plantations averaged 4.95 m^3/ha. RAM accounted for 50% of this volume loss. Approximately 18% of total mortality was stem breakage that occurred at stem galls. A larger proportion of RAM stem breakage, wind breakage, and windthrow occurred in thinned plots than in nonthinned plots. No rust-free trees were killed by adverse weather in nonthinned plots. Unknown causes were associated with 17% of the volume loss in thinned plots compared to 7% in nonthinned plots.

RAM was closely related to rust severity and competitive crown position in the stand (Table 3). High-risk trees accounted for 89% of RAM in nonthinned plots compared to 57% in thinned plots. Small trees, which had been marked but not cut by harvesting crews, accounted for most of the high-risk mortality in thinned plots. The average dbh of RAM trees in thinned plots was 10.9 cm compared to 13.0 cm in nonthinned plots.

Suppressed and intermediate trees represented approximately 80% of total RAM. Nance et al. (1981) found that RAM in Mississippi and Louisiana slash pine plantations was also greater among short trees than among tall trees. Severe stem infections appear to compound stress caused by competition among trees for moisture, nutrients, and light.

Probability estimates for the mortality of slash pine trees in nonthinned plots are shown in Table 4. Mean probability estimate for all trees during the 5-year study period was 0.22, with estimates ranging from 0.85 for high-risk suppressed trees to 0.00 for moderate-risk dominant trees. Probability of death within crown classes increased from 0.01 for dominant trees to 0.82 for suppressed trees. Rust severity also influenced probability estimates with values ranging from 0.37 for high-risk trees to 0.03 for low-risk trees. In general, dominant and codominant trees sustained minor losses in the 10 study plantations. Most of the losses occurred in suppressed and high-risk intermediate trees. These findings support the proposal by Lloyd (1982) that the ability to predict RAM can be improved by including a measure of stem-gall severity, rather than just the occurrence of rust.

Table 1. Average stand characteristics of thinned and nonthinned portions of 10 slash pine plantations infected with fusiform rust

Variable	Unit	Thinned			Nonthinned
		Initial	Removed	Residual	
Live trees	No./ha	1080	348	732	998
Basal area	m^2/ha	22.1	6.8	15.3	22.1
Volume	m^3/ha	161.9	51.2	110.7	158.6
Dbh	cm	15.5	15.0	15.7	16.3
Height	m	14.4	14.2	14.5	14.6

Table 2. Causes and relative occurrence of mortality in nonthinned and thinned portions of 10 slash pine plantations following the selective removal of rust-infected stems

Cause	Mortality			
	Nonthinned		Thinned	
	m^3/ha	%	m^3/ha	%
Fusiform rust				
RAM	9.53	61	1.53	31
RAM stem breakage	2.81	18	0.94	19
Total rust	12.34	79	2.47	50
Rust-free				
Suppression	0.63	4	0.44	9
Climatic	0	0	0.50	10
Insects	1.41	9	0.40	8
Logging	--	--	0.15	3
Other	0.16	1	0.15	3
Unknown	1.09	7	0.84	17
Total	15.63	100	4.95	100

Table 3. Percentage of rust-associated mortality by risk class and crown class in thinned and nonthinned portions of 10 slash pine plantations

Category	Rust-associated mortality (%)	
	Nonthinned	Thinned
Risk class		
High	89	57
Moderate	8	26
Low	3	17
Crown class		
Dominant	1	4
Codominant	16	18
Intermediate	57	44
Suppressed	26	34

Table 4. The influence of risk class and crown class on the probability of rust-associated mortality in nonthinned portions of 10 slash pine plantations

Risk class	Crown class				
	Dominant	Codominant	Intermediate	Suppressed	Total
High	0.036	0.151	0.496	0.847	0.368
Moderate	0.000	0.023	0.211	0.545	0.068
Low	0.013	0.011	0.163	--	0.030
Total	0.014	0.080	0.416	0.820	0.218

Table 5. Average stand characteristics of thinned and nonthinned portions of 11 loblolly pine plantations infected with fusiform rust

Variable	Unit	Thinned			Nonthinned
		Initial	Removed	Residual	
Live trees	No./ha	1171	311	860	1191
Basal area	m^2/ha	29.4	6.4	23.0	28.9
Volume	m^3/ha	206.7	43.5	163.2	201.4
Dbh	cm	17.5	16.0	18.0	17.0
Height	m	15.1	14.6	15.3	15.0

Loblolly Pine

The selective thinning of rust-infected stems in 11 loblolly pine plantations removed an average of 311 trees per hectare (Table 5). Residual stocking in the thinned plots averaged 860 trees per hectare compared to 1191 trees per hectare in the nonthinned portions of the plantations. The levels of thinning for individual plantations were classified as moderate to light. In general, average stocking levels were higher for loblolly pine than slash pine in both thinned and nonthinned portions of the study plantations.

Total volume loss for mortality in the nonthinned plots was 11.89 m^3/ha (Table 6). RAM accounted for 75% of these losses. Suppression, insects, and unknown factors caused most of the mortality of rust-free trees.

Total mortality in the thinned plots was 6.96 m^3/ha. RAM accounted for 44% of these losses. RAM stem breakage in loblolly pines was minimal in both thinned and nonthinned plots. Slash pine experienced significantly more RAM stem breakage than loblolly pine. Three factors account for the differences:

1. degree of stem infection and cankering was greater for slash pine than loblolly pine,
2. stem wood in slash pine is more brittle than in loblolly pine, and
3. sparse stocking in the slash pine plantations is conducive to wind-related stem bending and breakage concurrent with stem galls. Dense stocking minimizes breakage caused by wind or ice storms (Belanger and Brender 1968; Shepard 1978).

Mortality due to unknown causes represented 21 and 17% of total losses in thinned portions of loblolly and slash pine plantations, respectively. These losses are of particular concern from a forest management standpoint. Soil compaction and root breakage caused by logging equipment may account for some of the unknown mortality (Nebeker et al. 1983). Root rot fungi may be a second factor. No mortality has been attributed to root diseases during the initial 5-year study period; these assessments were based solely on above-ground signs and symptoms. Root excavations, isolations, and identification of root rot fungi will be conducted in all study plantations during the 6- through 10-year study period.

High-risk trees represented 79% of RAM in nonthinned loblolly plots compared to 62% in thinned plots (Table 7). Trees in the lower crown canopy are the most likely to die from rust. Suppressed and intermediate trees accounted for 90% of RAM. Loblolly pine sustained fewer losses in the dominant and codominant crown classes than did slash pine.

The influence of risk class and crown class on the probability of RAM for unthinned loblolly pine plantations is shown in Table 8. Probability of mortality within crown classes for trees with stem infections increased from 0.01 for dominant trees to 0.87 for suppressed trees. These trends in mortality related to crown classes are in agreement with findings reported by Shoulders and Nance (1987) for loblolly pine plantations in Mississippi and Louisiana. The probability estimate for mortality of high-risk trees was 0.42 compared to 0.02 for low-risk trees. Mean probability for all loblolly pine trees was 0.16 compared to 0.22 for slash pines. Most of the mortality (78%) occurred in suppressed and high-risk intermediate trees. Fusiform rust was associated with little mortality in dominant and codominant trees, regardless of rust severity.

Table 6. **Causes and relative occurrence of mortality in nonthinned and thinned portions of 11 loblolly pine plantations following the selective removal of rust-infected stems**

	Mortality			
	Nonthinned		Thinned	
Cause	m^3/ha	%	m^3/ha	%
Fusiform rust				
RAM	8.44	71	2.86	41
RAM stem breakage	0.48	4	0.21	3
Total rust	8.92	75	3.07	44
Rust-free				
Suppression	0.95	8	0.69	10
Climatic	0.36	3	0.69	10
Insects	0.59	5	0.21	3
Logging	--	--	0.07	1
Other	0.24	2	0.77	11
Unknown	0.83	7	1.46	21
Total	11.89	100	6.96	100

Table 7. **Percentage of rust-associated mortality by risk class and crown class in thinned and nonthinned portions of 11 loblolly pine plantations**

	Rust-associated mortality (%)	
Category	Nonthinned	Thinned
Risk class		
High	79	62
Moderate	16	26
Low	5	12
Crown class		
Dominant	1	2
Codominant	8	9
Intermediate	53	44
Suppressed	38	45

Table 8. The influence of risk class and crown class on the probability of rust-associated mortality in nonthinned portions of 11 loblolly pine plantations

	Crown class				
Risk class	Dominant	Codominant	Intermediate	Suppressed	Total
High	0.000	0.069	0.487	0.882	0.418
Moderate	0.011	0.026	0.236	0.722	0.094
Low	0.006	0.009	0.107	1.000	0.020
Total	0.007	0.026	0.349	0.868	0.163

SUMMARY

Five-year results following the selective thinning of slash and loblolly pines with fusiform rust stem galls show:

1. rust-associated mortality in nonthinned study plots is significantly greater in slash pine than in loblolly pine,

2. a large percentage of RAM is in intermediate and suppressed trees with severe stem infections,

3. selective thinning can reduce RAM in merchantable slash and loblolly pine plantations, and

4. gains in total volume salvaged by thinning were 10.68 m^3/ha for slash pine and 4.93 m^3/ha for loblolly pine.

Mortality is but one factor being evaluated in testing silvicultural options for managing merchantable pine plantations infected with fusiform rust. Total volume yields, stand improvement, and product value are being considered as well.

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PRELIMINARY ASSESSMENT OF GROWTH COMPENSATIONS IN SLASH PINE AND LOBLOLLY PINE PLANTATIONS WITH SIMULATED FUSIFORM RUST MORTALITY

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ABSTRACT

Pine trees with fusiform rust caused by the fungus *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* often die or probably suffer reduced growth, but in time the surviving trees may benefit from the additional growing space. A thinning study was initiated in south Mississippi to estimate growth compensations that are likely to occur in stands with mortality caused by fusiform rust. The two sites selected for study had less than 5% stem infection at age 5 when thinning treatments were imposed. Zero, 25%, or 50% of the trees were randomly removed from 90 plots to simulate different levels of fusiform rust mortality. Percentages of trees removed each year were patterned after mortality observed in plantations with high incidence of rust. Preliminary results indicate that loblolly pine (*Pinus taeda* L.) and slash pine (*P. elliottii* Engelm. var. *elliottii)* trees respond to reduced stand density as early as the eighth or ninth growing seasons, but it is too early to predict how much growth compensation is likely to occur over long rotations for pulpwood or sawtimber.

INTRODUCTION

Fusiform rust caused by the fungus *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme* is a major disease of slash pine (*Pinus elliottii* Engelm. var. *elliottii*) and loblolly pine (*P. taeda* L.) in the southern United States. Most of the potentially damaging infections develop during the first five growing seasons after trees are planted (Froelich and Snow 1986; Froelich 1989).

In stands with fusiform rust, tree mortality is probably the major factor reducing productivity, but infected trees that survive to rotation may also have reduced growth. However, some of these losses may be compensated for, in time, by the surviving trees whose diameter growth is benefited by the reduced competition among trees for growing space. Such compensation seems likely for stands damaged by fusiform rust because southern pines are often planted at densities of about 1700 trees per hectare, leading to early competition for light, water, and nutrients. A high percentage of the rust-associated mortality often occurs by age 10, leaving many years for the remaining trees to compensate for the early losses.

Assessing compensations is difficult because the growth of each tree in the forest--whether infected or rust free--is governed by the sizes and spatial relationships with neighboring trees. This interdependence in growth among trees may make it impossible to separate the compensations from other growth components, particularly from the growth reductions that are likely to occur in infected trees. Consequently, I believe that growth compensations can be best evaluated with thinning experiments that simulate fusiform rust mortality as closely as practical. This report gives preliminary results of such an experiment.

METHODS

The fusiform rust mortality simulation study was established in two 5-year-old pine plantations with low incidence of fusiform rust. The two plantations (Locations 4 and 5) were part of a controlled experiment designed to study the impact of fusiform rust on forest productivity (Froelich 1987). The two sites had been plowed and disked, and then planted by hand in February of 1978 or 1979 with 1-year-old, nursery-grown slash pine or loblolly pine seedlings. The same seed lots of slash pine and loblolly pine had been planted in both years at each location, and the plots had been mowed annually.

There were eighty-two 80-tree plots. Spacing was 1.8 m between trees within rows and 3.0 m between the eight rows of trees in each plot. Establishment density was constant among plots because two trees had been planted about 20 cm apart and the smallest survivor had been removed at the beginning of the second season of growth. The plots had been set out in random block arrangement, normally with five plots of either slash pine or loblolly pine to a block. There were two blocks of each species-age class at each location.

The study plan called for artificial inoculation of trees in some plots; however, the inoculation of trees with suspensions of basidiospores was not very successful. At age 5, only 4 of 82 plots had developed more than 10% fusiform rust stem infection (5% infection overall at Location 4, and 2% at Location 5). Since the study plots were therefore no longer suitable for assessing effects of rust on yield, I redesigned the experiment so the 82 plots could be used as a thinning study for estimating growth compensations in stands with trees killed by fusiform rust. The low incidence of rust made the plots ideal for a compensation experiment because tree growth after thinning would not be confounded by possible reduced growth of infected trees. Another desirable feature was that most plots still contained 70 to 80 trees at age 5.

Three mortality simulation treatments were imposed: 1) one called for random removal of 20 trees (25% of the established trees) per plot, including natural mortality; 2) one called for removal of 40 trees; and 3) one treatment designated no trees for removal.

Tree height measurements at age 5 sometimes revealed considerable variation in the apparent site quality of the five plots comprising the individual blocks. Very large variation in apparent site quality was even apparent within several individual plots, of both slash pine and loblolly pine, planted in 1978 at Location 4. Due to diversity of apparent site quality within each species-age class, and the small number of plots in each class, it was not possible to block the experiment so that each block would contain three plots (treatments) of similar apparent site quality, as defined by mean heights of dominant and codominant trees at age 5. Therefore, plots that were similar in apparent site quality were paired, and the 25 or 50% treatments were assigned randomly to each pair. No thinning was done on the remaining plots, providing an array of density-site combinations for evaluating growth compensations.

Fusiform rust mortality patterns in two slash pine plantations in south Mississippi provided a guide for timing the tree removals in the mortality simulation plots (Fig. 1). The plantations used to construct the observed rust mortality patterns in Figure 1 had developed about 50% fusiform rust stem infection by age 5. As indicated, few trees had died from rust before age 5, and the rust-related mortality was tapering off considerably by the tenth growing season when the thinning schedule was developed for the mortality simulation experiment (subsequent mortality in year 12 was due mainly to hurricane Elena). Since a common procedure in the southern United States is to thin pine plantations of rusted trees at about age 15, future rust-related mortality was estimated through age 15, and percentage mortality was computed by year. These percentages were multiplied by 20 or 40 to determine the number of trees to be removed

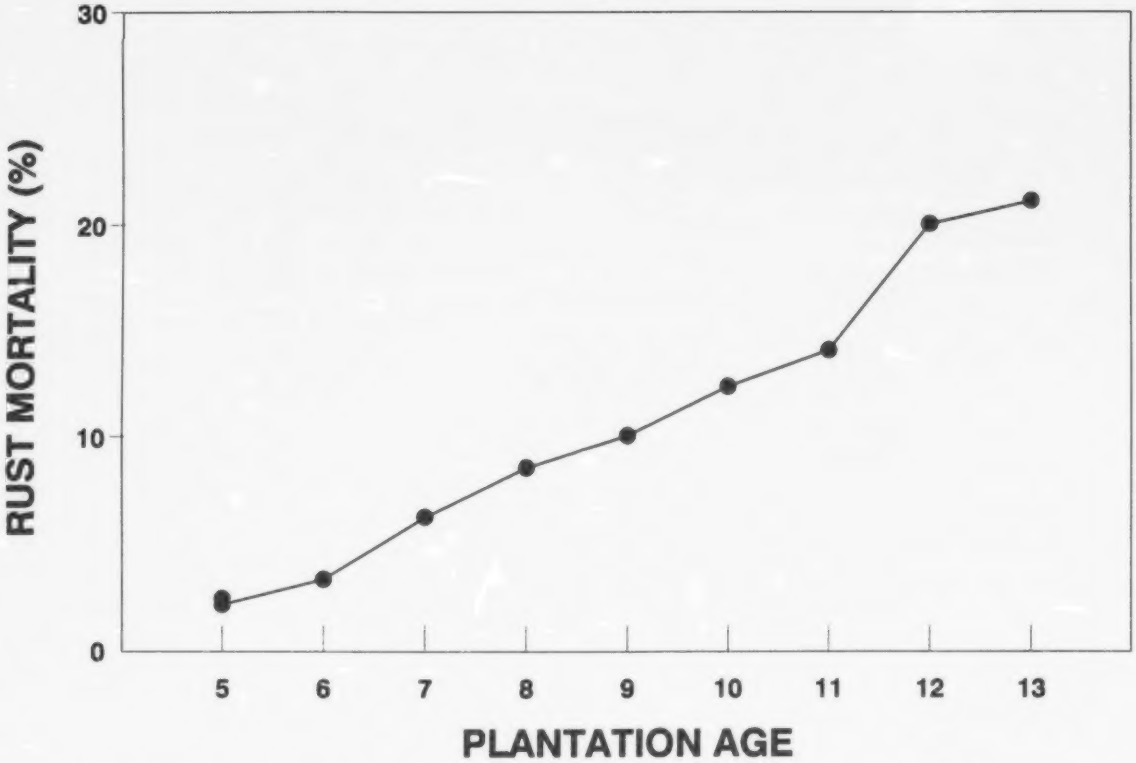

Figure 1. Cumulative fusiform rust mortality observed in two slash pine plantations in south Mississippi, with about 50% stem infection at age 5.

each year in the two simulation treatments. To simplify the experiment, mortality computed for the tenth through fifteenth seasons was allocated to the end of the ninth growing season. The percentages of trees actually removed each year were adjusted to accommodate natural mortality. Also, when hurricane Elena struck the plots in 1985, further removals were postponed to ensure that total mortality would not exceed 25 or 50% at the end of the ninth growing season.

Although fusiform rust infections are randomly distributed among trees in the forest (Webb et al. 1986), a restricted randomization procedure was developed for selecting trees for removal. The procedure ensured that exactly 25 or 50% of the trees would be removed, or would die of natural causes, in each quarter plot of 20 established trees. This prevented creation of very large undesirable pockets of missing trees in some plots.

Since within-plot site differences were still pronounced in nine plots when analysis was contemplated for this paper, the nine plots were divided into 17 subplots, with 40 or more trees per subplot, but with uniform site quality within the new plot boundaries. Two subplots were in a 25% simulation plot, and the rest were in unthinned plots. Thus, there were 90 plots (including subplots) available for analysis after measurements were made at age 10 or 11--the end of the 1988 growing season. Forty-nine plots were simulation plots containing about 50 or 75% of their original number of trees, or current equivalents of 900 or 1345 trees per hectare. Most of the mortality was simulated, but some trees had died of natural causes, including a few trees of fusiform rust. One unthinned subplot of poor site quality had relatively high natural mortality and now contains 1255 trees per hectare; 37 of 41 unthinned plots still have more than 90% of their original 1794 established trees.

Regression analyses were used to assess growth compensations. The dependent growth variables to be predicted--basal area growth per tree in different years--were based on tree diameter measurements made 137 cm above the ground at ages 5, 7, 9, 10, and 11. These diameter measurements were converted to area growth per tree (per year) for the years 6 and 7, 8 and 9, 10, and 11. Because only the plots planted in 1978 have completed the eleventh season of growth, regressions based on age-11 data will not be given, although growth trends through age 11 will be displayed in some graphic summaries.

It was hypothesized that, in the early growing seasons, average growth per tree should be a function of only the site quality of the land, as expressed by the mean height of dominant and codominant trees preceding the growth period. The addition of a second independent variable to the regression equation--number of trees per hectare--was not expected to improve the prediction of growth in early years because individual trees in all plots should then have optimum space for growth, and stand density would still be almost constant among plots. It was further hypothesized that when trees in plots with reduced density respond to the additional growing space, the response should be reflected by increasing importance of the independent variable "number of trees per hectare," either as a single predictor variable of growth per tree or as a second independent variable in the regression equations. A negative beta coefficient for the density variable would further confirm that trees in plots with reduced density were growing faster than trees in unthinned plots.

Separate regressions were developed for slash pine and loblolly pine, by location, and by sequential growth periods. The data from 1978 and 1979 plantings were combined, and regressions for each period of growth were therefore based on data from 20 to 26 plots with varying densities.

To ensure that the regressions for sequential periods of growth would not be affected by an ever-changing population of living trees, study of growth in consecutive years was based on the same trees: the trees were alive at age 10 or age 11, at least 5 cm in diameter at age 10 or 11, and free of top breakage or fusiform rust that could bias the basal area growth trends.

RESULTS

Results of 36 regressions are given in Table 1. As hypothesized, regressions show that biennial basal area growth per tree for the sixth and seventh growing seasons is best expressed by the single predictor variable "mean height of dominant and codominant trees at age 5" (DCH5). Number of trees per hectare at age 5 (N5) has no value for predicting this early growth, either as a single predictor variable or as an added variable in the regression equations. For example, at Location 4 the mean height of dominant and codominant trees at age 5 produces a coefficient of determination (R^2) of 82.4% for slash pine and 97.6% for loblolly pine. The addition of N5 improves the prediction of basal area growth during years 6 and 7 slightly for slash pine (R^2 = 84.2), but not at all for loblolly pine. N5 does not serve as a useful single predictor of basal area growth for the sixth and seventh growing seasons for either species (R^2 = 0.4 and 4.3%, respectively, for slash pine and loblolly pine in Location 4). However, in the eighth and ninth growing seasons, or tenth growing season, growth compensations seem apparent in both slash pine and loblolly pine. The addition of number of trees per hectare improves the prediction of area growth by 14.5 to 30.7% for slash pine and by 5.0 to 8.2% for the loblolly pine for the growth periods 8 and 9,

Table 1. Regression analyses for predicting basal area growth per tree (Y) after experimental plots were thinned to simulate fusiform rust mortality. Independent variables (X_1,X_2) used to predict annual or biennial growth are mean height of dominant and codominant trees (DCH and specified year) and number of trees per hectare (N and specified year)

Y	X_1	X_2	R^2 (%)	Y	X_1	X_2	R^2 (%)
Location 4--Slash pine				Location 5--Slash pine			
6 + 7[a]	DCH5	--	82.4	6 + 7	DCH5	--	88.5
6 + 7	DCH5	N5	84.2	6 + 7	DCH5	N5	88.5
6 + 7	N5	--	0.4	6 + 7	N5	--	11.1
8 + 9	DCH7	--	54.9	8 + 9	DCH7	--	14.0
8 + 9	DCH7	N7	69.4	8 + 9	DCH7	N7	34.9
8 + 9	N7	--	28.3	8 + 9	N7	--	19.2
10	DCH9	--	36.9	10	DCH9	--	12.2
10	DCH9	N9	67.6	10	DCH9	N9	34.0
10	N9	--	44.6	10	N9	--	22.1
Location 4--Loblolly pine				Location 5--Loblolly pine			
6 + 7	DCH5	--	97.6	6 + 7	DCH5	--	88.5
6 + 7	DCH5	N5	97.6	6 + 7	DCH5	N5	89.3
6 + 7	N5	--	4.3	6 + 7	N5	--	4.8
8 + 9	DCH7	--	86.6	8 + 9	DCH7	--	4.1
8 + 9	DCH7	N7	91.6	8 + 9	DCH7	N7	12.4
8 + 9	N7	--	10.4	8 + 9	N7	--	3.4
10	DCH9	--	81.2	10	DCH9	--	1.2
10	DCH9	N9	89.4	10	DCH9	N9	8.3
10	N9	--	23.3	10	N9	--	4.8

[a] Basal area growth (Y) for the sixth and seventh growing seasons.

and 10. All beta coefficients for the density variable were negative after the sixth and seventh growing seasons. At Location 5, a poor site for growing loblolly pine, basal area growth of loblolly pine has not been predictable after the seventh growing season.

Basal area growth trends in Figure 2 tend to corroborate the results of the regression tests. These six sets of curves (A to F) stratify the data according to similarities in mean heights of dominant and codominant trees at age 9 and show trends in the average basal area growth per tree (per year). Individual curves are based on data from two or three plots; the same trees used in regression analyses were used to compute average basal area growth per tree. Sets A and B for slash pine at Location 4 suggest that trees began to grow faster in the 50%-simulation plots as early as the eighth and ninth growing seasons. As with the regressions, response is less clear for other sets, although some compensation may be occurring in years 10 or 11. Growth response is not apparent in any of the 25%-treatment plots.

Figure 3 shows basal area trends of representative plots, expressed in square metres of basal area per hectare (or square feet per acre). Differences in basal area trends of plots with about the same number of trees per hectare, (for example, plot 19 versus plot 22) are due to variations in site quality among plots, as expressed by average heights of dominant and codominant trees. Since basal area growth increases have been small compared to the volume of trees removed by thinning, effects of thinning are not readily apparent on a forest stand basis.

CONCLUSIONS

Regression analyses and plotting of the data indicate that trees in some plots with simulated fusiform rust mortality responded very quickly to the extra growing space, apparently as early as the eighth and ninth growing seasons. The regression analyses suggest that the growth response was more immediate in slash pine than in loblolly pine, but the distinction between species was not clear when the growth data were plotted in Figure 2. The difficulty in predicting basal area growth of loblolly pine in Location 5 may be related in part to poor height and diameter growth in most plots. There is a narrow range in apparent site quality among the 20 plots of loblolly in this location. Combining the two age classes of data for regression analyses may also be confounding growth relationships. To illustrate, the plots established in 1978 (A, C, and F, in Fig. 2) usually have ascending growth curves in early years while identical plots established in 1979 (B, D, and E) have decreasing early trends. These differences are indicative of climatic variations that need to be considered in future analyses.

Apparent growth response as early as the eighth and ninth growing seasons was surprising because about 45% of the trees were removed at the end of the ninth season of growth (about 45% had been removed after years 6 or 7, and 10% after year 8). However, early diameter growth response is consistent with results of Ginn and others (1989). The loblolly pine plots in their experiment had been planted at 3.0 × 3.0 m spacing and thinned by removing alternate rows of trees at age 8. During the first growing season after thinning, trees in thinned plots grew more in live crown diameter, diameter at breast height, crown surface area, and dry weight than did trees in unthinned plots. Trees in unthinned plots grew more in height, however.

It is too early to predict how the apparent growth responses will affect future volume yield per hectare in the various treatments; large increases in growth will have to continue for several years to compensate significantly for the current low volumes in plots with simulated rust mortality (Fig. 3). Future assessments will consider effects of the thinnings on both tree height growth and tree taper.

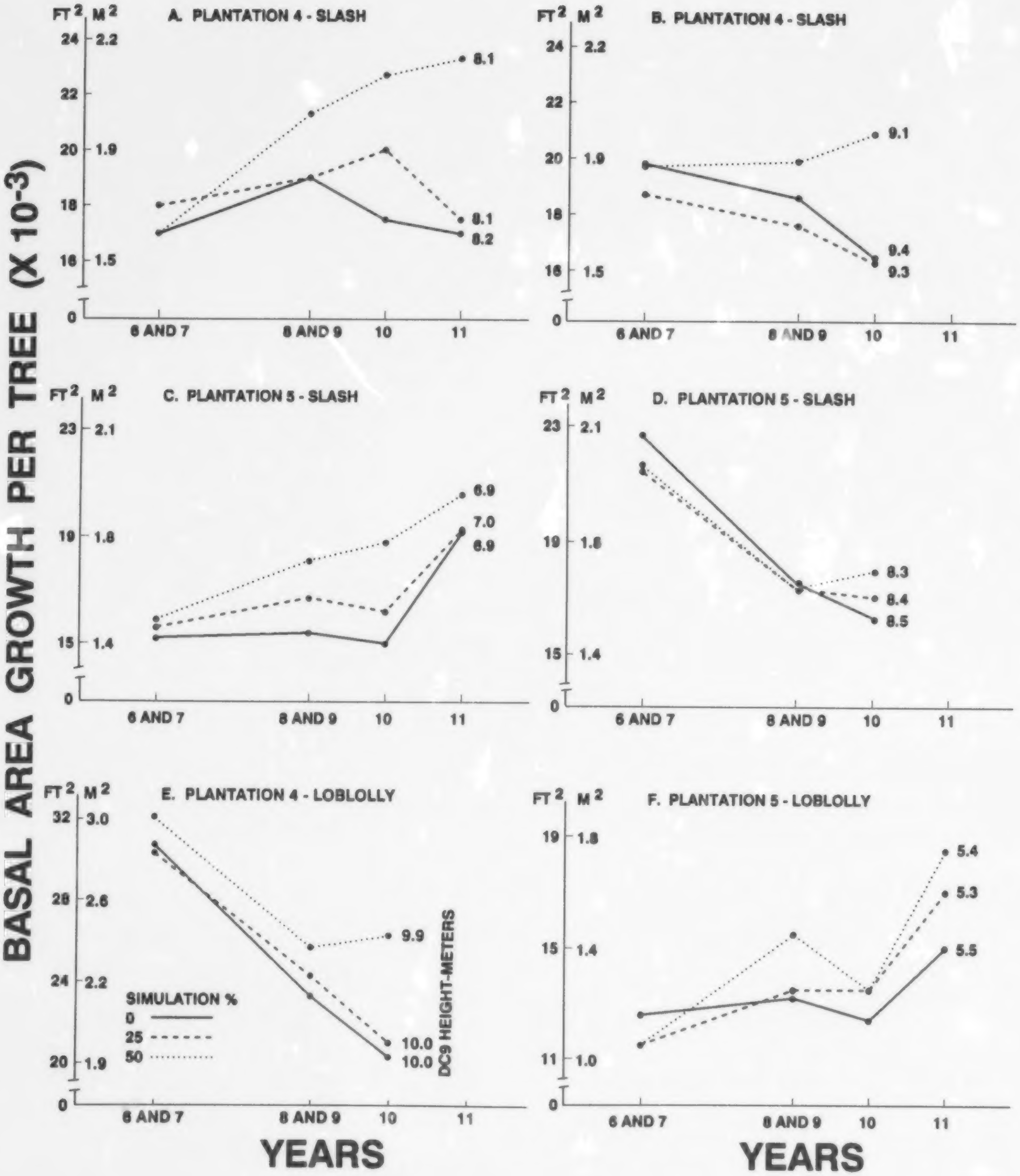

Figure 2. Basal area growth per tree (per year) in plots with simulated fusiform rust mortality. Each curve in A to F was based on data from two or three plots of similar site quality, as expressed by average heights of dominant and codominant trees at age 9 (DC9).

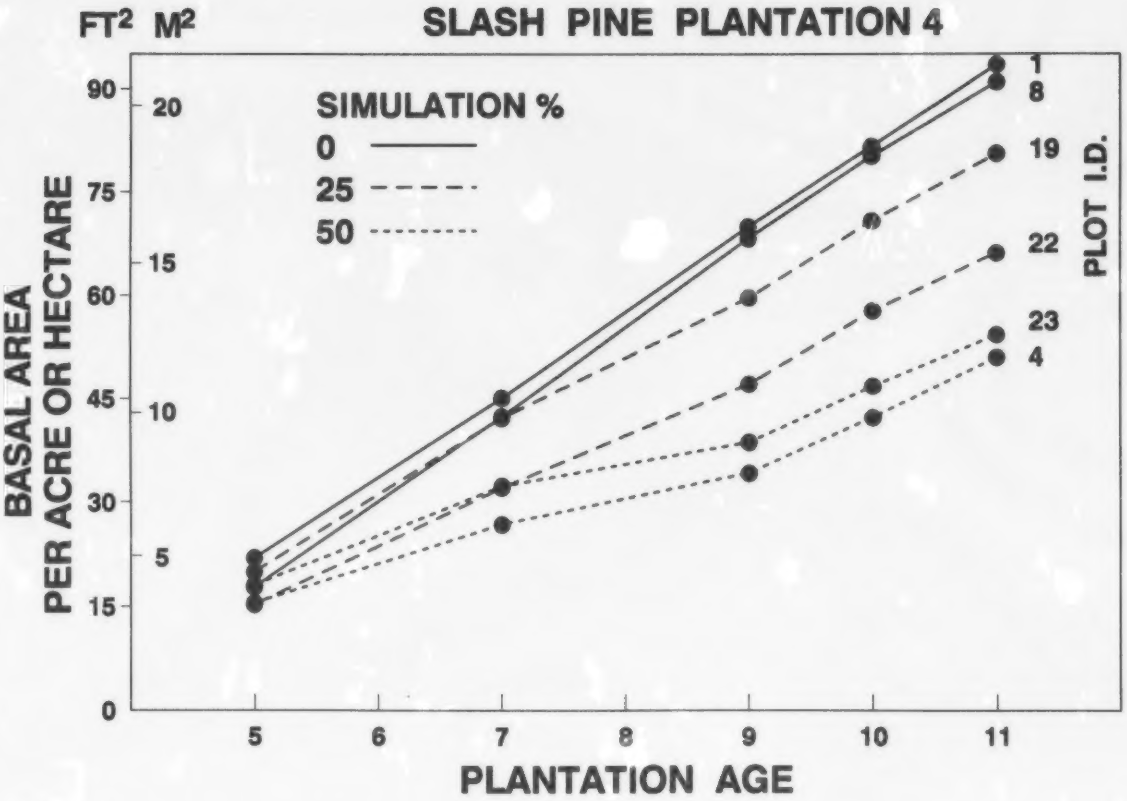

Figure 3. Basal area growth trends (per acre or hectare) of representative plots on which different percentages of trees were randomly removed to simulate fusiform rust mortality.

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FUSIFORM RUST AECIOSPORE PRODUCTION AFTER THINNING IN *PINUS ELLIOTTII* VAR. *ELLIOTTII* AND *PINUS TAEDA* PLANTATIONS: A 5-YEAR ASSESSMENT

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The fusiform rust disease epidemic in the southern United States is fueled by complex interactions of biological and environmental factors. Among these factors are weather, sporulation and spore dispersal, populations of susceptible hosts, and the presence of pine and oak inoculum produced by the causal fungus *Cronartium quercuum* (Berk.) Miyabe ex Shirai f. sp. *fusiforme*. The biological components have been altered and the disease exacerbated by forest management practices designed to increase the growth and yield of slash (*Pinus elliottii* Englem. var. *elliottii*) and loblolly (*P. taeda* L.) pines. Large numbers of these two susceptible pine species have been and are being planted in areas with populations of highly susceptible oak (*Quercus* sp.) species. Frequently these plantings are adjacent to diseased pine stands that provide abundant inoculum potential for infection of oak leaves and, subsequently, the young planted pines. The pine plantations are usually established after intensive mechanical and chemical site preparation that promotes rapid early growth of the pines. The combination of aeciospore inoculum, dense populations of young and rapidly growing susceptible pines, and abundant oaks provides the potential to increase the rust hazard of a site.

A management strategy proposed to mitigate this disease cycle includes harvesting and regenerating pines in blocks so that adjacent plantations differ in age by 12 to 15 years (Schmidt et al. 1977). The underlying assumption of this strategy is that infected pine plantations older than 10 years are beyond the period of maximum aeciospore production and provide less inoculum for continuation or intensification of the disease.

Research has provided information on fusiform rust gall longevity and the phenology and dynamics of aeciospore production in slash pine from ages 1 through 5 (Griggs 1985) and in loblolly and slash pine plantations ranging in age from 7 to 17 years (Kuhlman 1981, Chappelka and Schmidt 1983, Chappelka et al. 1984). The number of living galls, number of sporulating galls, and the proportion of individual gall surface area producing aeciospores decreased with increasing age of plantations (Chappelka et al. 1984). Also, Chappelka and Schmidt (1983) found that the mean duration of aeciospore production decreased as plantations aged. The two major reported causes of reduction in the number of rust galls over time were mortality of trees with fusiform rust stem galls and death of infected branches in the lower crown due to shading and suppression following crown closure (Kuhlman 1981).

An additional reduction in aeciospore inoculum may be possible through the selective removal of trees with galls capable of producing aeciospores. Under present conditions, such a treatment would be economically acceptable only if the harvested stems could be converted into useable products, and the operation significantly increased growth and product value of the residual stand.

In 1981, we initiated a study to evaluate the potential of sanitation-salvage harvests in merchantable plantations of slash and loblolly pine ranging in age from 13 to 21 years. The principle objectives were to determine whether a sanitation-salvage thinning, based primarily on removal of fusiform rust infected stems, would: (1) alter the numbers of living galls with the potential to produce aeciospores, or (2) stimulate aeciospore production by residual galls that would counteract the reduction in gall numbers.

This report summarizes results 5 years after treatment, and discusses disease and forest management implications.

MATERIALS AND METHODS

The criteria for plantation selection and the details of plot installation and general data collection are described by Belanger et al. in these proceedings. Data were collected from eight 0.1-hectare plots in each of 10 slash pine and 11 loblolly pine plantations. Half the plots were in thinned and half in nonthinned portions of the plantations. Before thinning, diameter at breast height (dbh) was measured, stem and branch galls were counted, and a crown class and a rust risk class were assigned for each tree on all plots. Within each of the 0.1-ha plots, intensive rust measurements were then taken on a 20% sample of trees on the plots based on a representative range of diameters. These measurements included

1. numbers and heights of all stem galls (heights were from ground level to the gall midpoint),
2. estimated percentage of stem circumference galled,
3. estimated percentage of stem circumference of each gall that was cankered, and
4. number of living branch galls.

Fusiform rust data for the thinned and nonthinned portions of the study plantations are summarized in Table 1 for slash pine and in Table 2 for loblolly pine, and show the initial effects of thinning on rust incidence, numbers of galls, and gall characteristics. The initial distribution of stem galls by 1.52-m intervals from the ground for the two pine species, thinned and nonthinned plots combined, are illustrated in Figures 1A and 1B.

Data on fusiform rust gall sporulation were collected by observing the trees in the 20 percent subsample for five successive sporulation seasons starting with the first season after treatment. We timed our observations each year to coincide with the peak of aeciospore production. However, since galls were observed only once a year, we may have missed some late-sporulating galls. Data collected for stem galls included 1) gall sporulation, and 2) proportion of gall surface area producing aeciospores.

Stem galls on living trees were assumed capable of producing aeciospores and are termed living stem galls. Conversely, stem gall mortality was defined by death of the trees with stem galls. The

Table 1. **Mean fusiform rust characteristics of thinned and nonthinned portions of 10 *Pinus elliottii* var. *elliottii* plantations**

Variable	Unit	Thinned			Nonthinned
		Initial	Removed	Residual	
Stem infections					
Infected stems	No./ha	526	326	300	474
Incidence	%	49	94	27	49
Total stem galls	No./ha	919	637	282	837
Gall characteristics					
Circumference	% of stem	61	67	50	62
Cankering	% of stem	31	33	24	35
Branch galls	No./ha	440	188	252	380
Total galls	No./ha	1359	825	534	1217

Table 2. **Mean fusiform rust characteristics of thinned and nonthinned portions of 11 *Pinus taeda* plantations**

Variable	Unit	Thinned			Nonthinned
		Initial	Removed	Residual	
Stem infections					
Infected stems	No./ha	670	279	390	665
Incidence	%	58	90	46	56
Total stem galls	No./ha	1226	600	625	1213
Gall characteristics					
Circumference	% of stem	50	58	43	53
Cankering	% of stem	22	25	17	26
Branch galls	No./ha	425	163	262	501
Total galls	No./ha	1651	763	887	1714

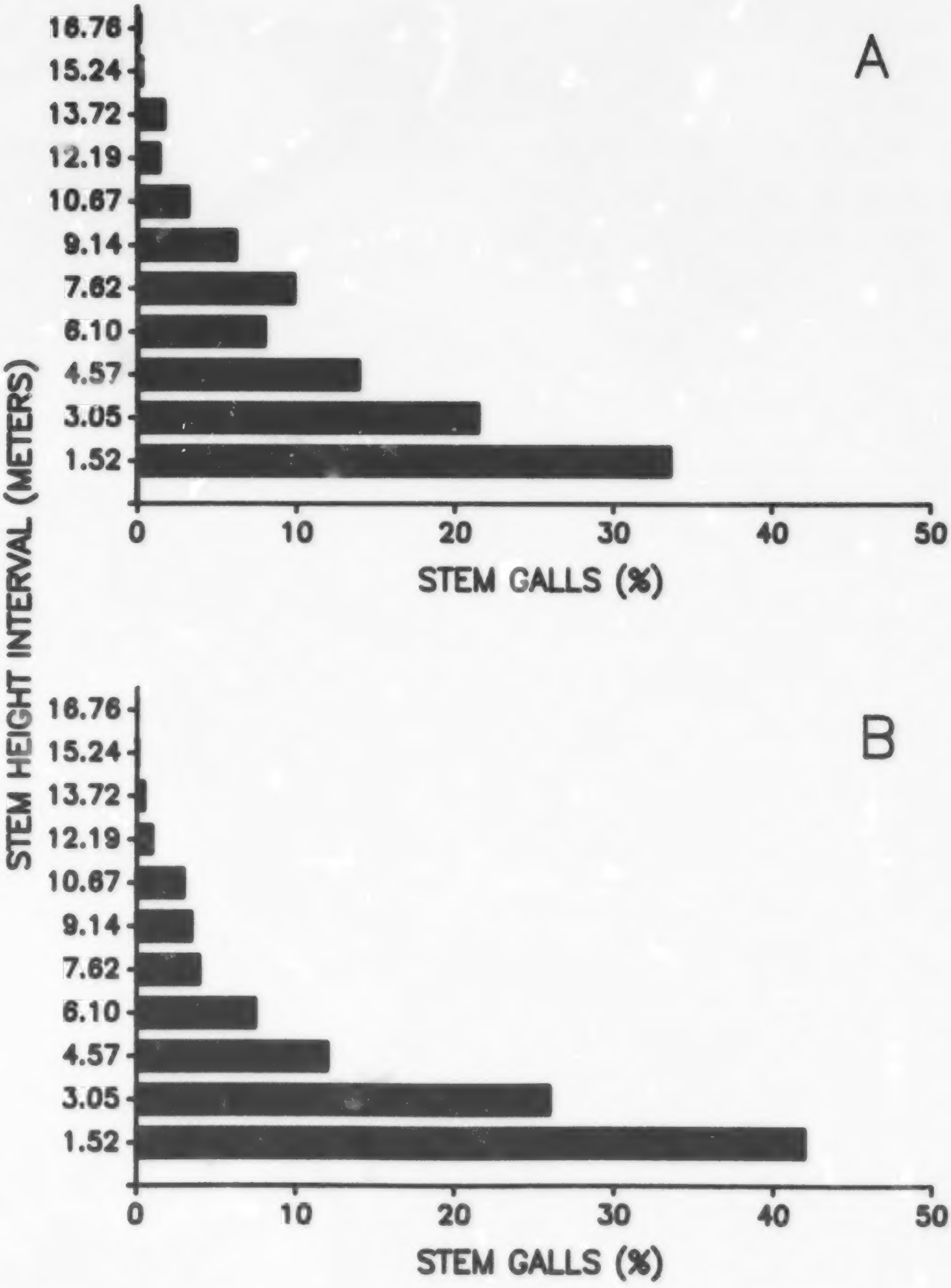

Figure 1. **Percentage of living stem galls of fusiform rust occurring in 1.52-m intervals on stems of (A)** *Pinus elliottii* var. *elliottii* and **(B)** *P. taeda* measured from the ground upward.

position of each stem gall was documented, allowing an accurate record of the yearly sporulation for each gall on each sample tree. For branch galls, however, such accuracy was impossible because infected branches died and new galls may have developed. Therefore, for branch galls, only the number of galls and the proportions producing spores were documented during each observation period. Because most of the sample trees were >12 m in height, binoculars were used to count and observe branch galls.

All sporulation data presented are mean values for each of the two host species across plantations and by treatment. Data expressed on a hectare basis are calculations from the total samples of trees within the 0.1-ha plots or from the 20% samples within these plots.

RESULTS

Thinning reduced the estimated number of branch and stem galls in the original stands by 61 and 46% for slash and loblolly pines, respectively. The greatest reduction was in the number of stem galls (Tables 1 and 2). Thinning also reduced the mean proportion of stem circumference encircled by galls and the degree of stem cankering in the residual stands of both species.

After treatment, the mean number of stem galls per hectare in the nonthinned portions of the slash pine plantations was nearly three times that in the thinned areas (837 vs. 282) (Fig. 2A). This difference remained nearly constant through the five sporulation seasons, with a decrease in number of galls of 28% for the nonthinned and 27% for the thinned areas. In loblolly pine, the initial number of stem galls per hectare in the nonthinned areas was almost double that in the thinned (1213 vs. 625) (Fig. 2B). The difference between the thinned and nonthinned areas of loblolly pine narrowed somewhat over 5 years because of a 25% decrease in numbers of galls in nonthinned areas compared to only a 7% loss in the thinned areas.

In four of the five sporulation seasons, a greater percentage of slash pine stem galls sporulated in nonthinned than in thinned areas (Fig. 3A). There was a general increase in percent sporulation from the first to the second season, followed by a decrease in the third season. The sporulation then remained relatively constant in the nonthinned areas but declined consistently in the thinned areas to a low of 7% in year 5.

Stem gall sporulation in loblolly pine was remarkably similar between the two treatments, averaging about 24% for the first 4 years, then declining to slightly less than 20% in the fifth season (Fig. 3B). There was a consistently higher percentage of stem galls sporulating on loblolly than on slash pine for all sporulation periods.

There was no difference between the frequency of sporulation of stem galls in thinned and nonthinned areas for either pine species. Slash pine galls sporulated an average of 1.5 times, while those of loblolly produced spores an average of 2 times, over 5 years.

The estimated number of branch galls per hectare recorded in the first spore season was greater in nonthinned than in thinned plots for both species, 1.5 and 1.9 times for slash and loblolly pine, respectively (Figs. 4A and 4B). This trend continued through the 5 years. The number of galls per hectare decreased dramatically between years 1 and 5, regardless of treatment. In the fifth year we recorded a mean of fewer than 50 branch galls per hectare for both species and treatments. This represented a maximum decrease of about 463 branch galls per hectare for nonthinned loblolly pine and a minimum decrease of 229 galls per hectare for thinned slash pine (Figs. 4A and 4B).

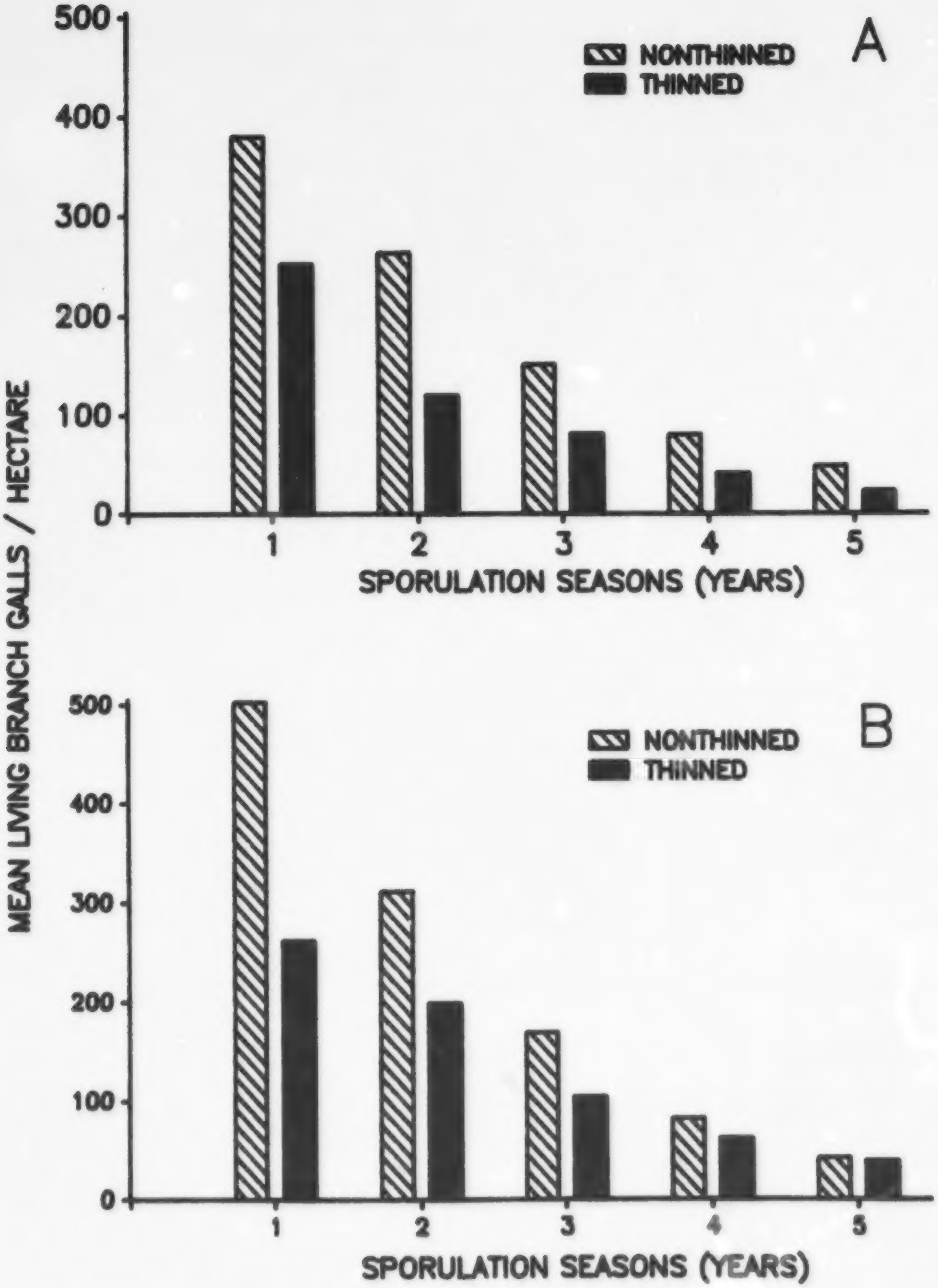

Figure 2. **Mean number of living stem galls per hectare of fusiform rust in thinned and nonthinned portions of (A)** 10 plantations of *Pinus elliottii* var. *elliottii* and **(B)** 11 plantations of *P. taeda* during five successive aeciospore seasons (years).

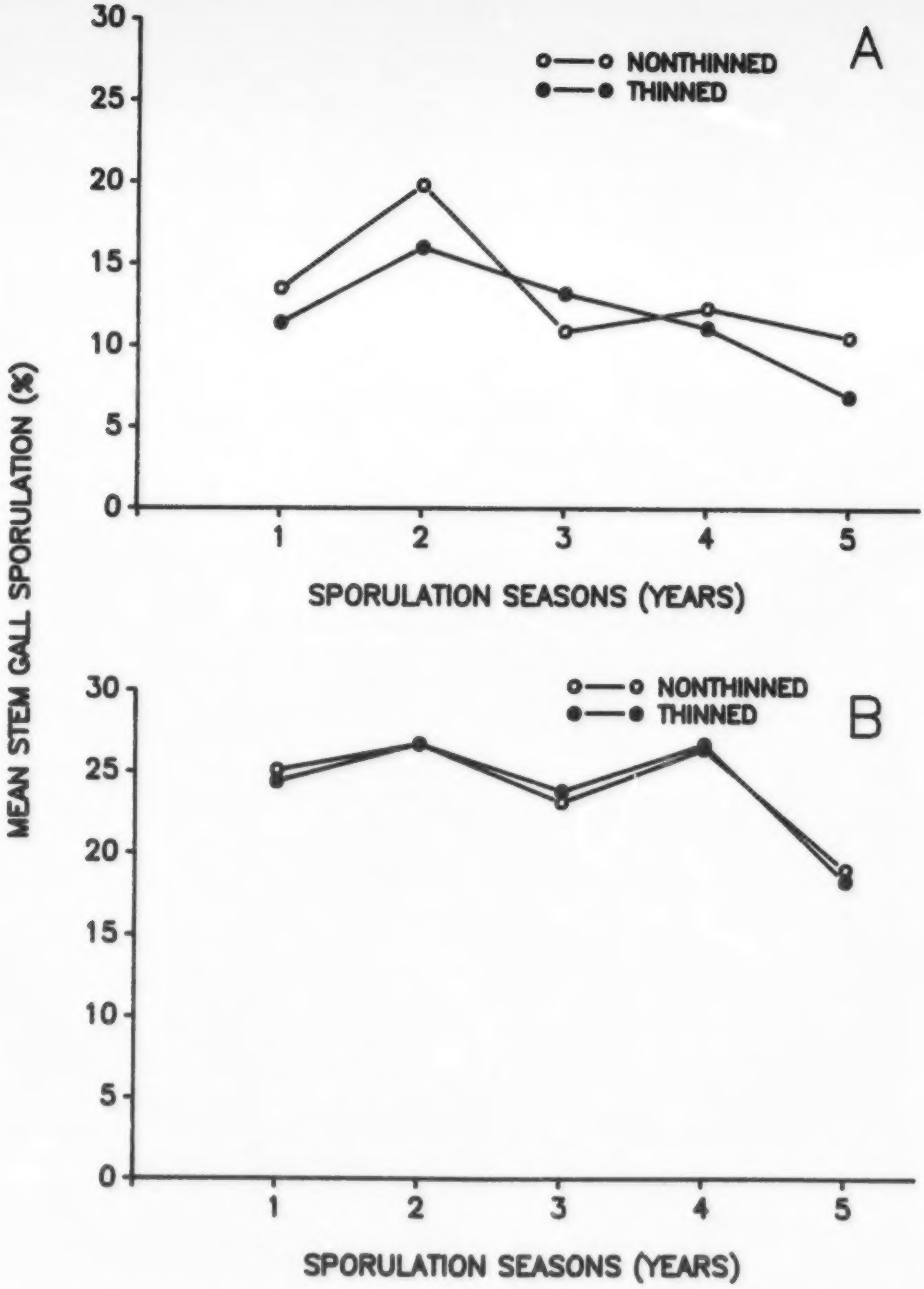

Figure 3. Mean percentage of living stem galls of fusiform rust that produced aeciospores in thinned and nonthinned portions of (A) 10 plantations of *Pinus elliottii* var. *elliottii* and **(B)** 11 plantations of *P. taeda* during five successive sporulation seasons (years).

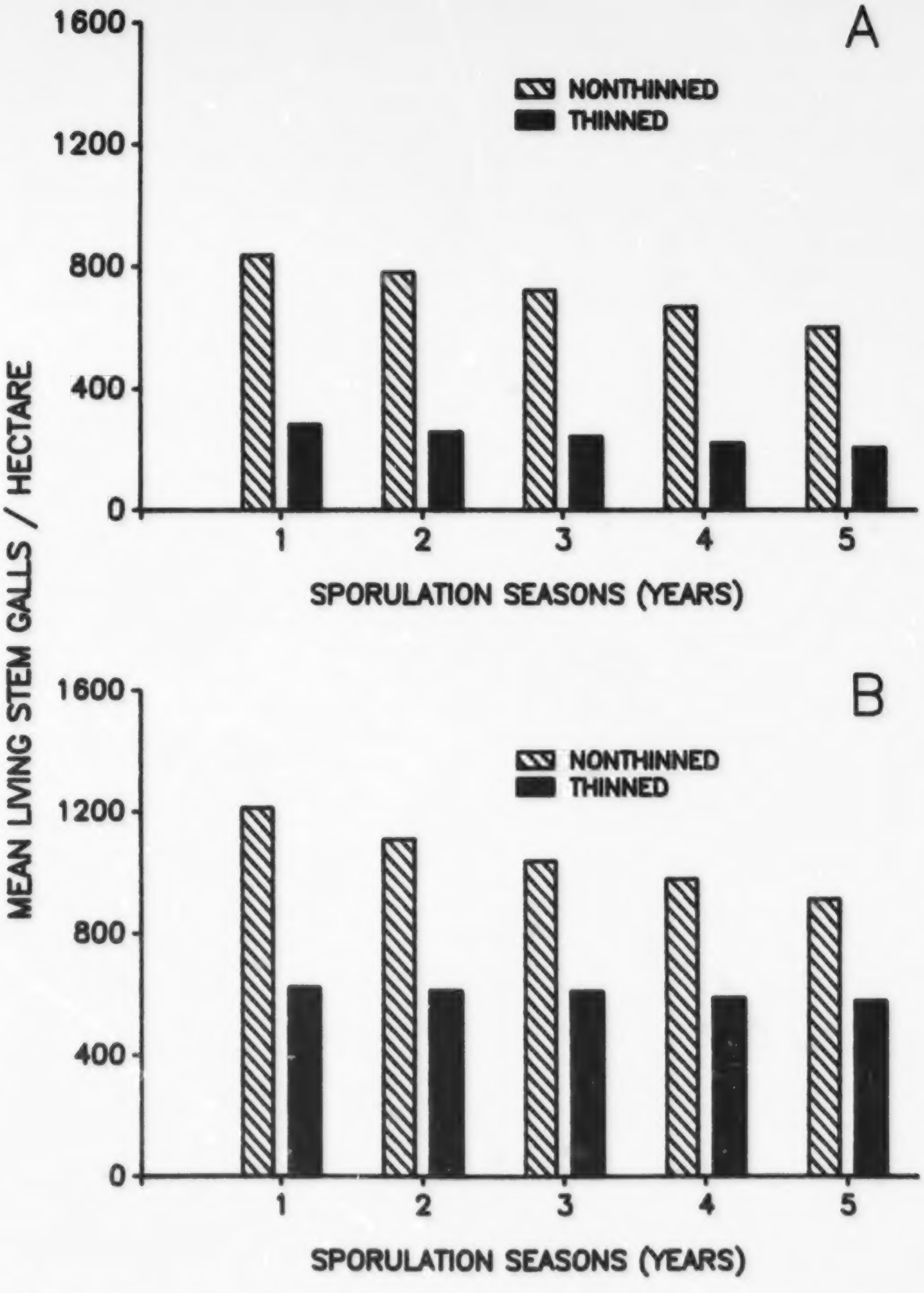

Figure 4. Mean percentage of living branch galls per hectare of fusiform rust in thinned and nonthinned portions of (A) 10 plantations of *Pinus elliottii* var. *elliottii* and **(B)** 11 plantations of *P. taeda* during five successive aeciospore seasons (years).

The mean percentage of branch galls of slash pine that sporulated decreased in each of the 5 years of observation, with the exception of a minor increase in the thinned areas in year 5 (Fig. 5A). In the loblolly pine plantations, sporulation varied considerably between years (Fig. 5B), with major increases occurring in the thinned areas in years 1 and 3.

The changes in the estimated total number of living galls per hectare and percentage of gall mortality over the 5-year observation period are shown in Table 3 for both species. The percentage decrease in total galls per hectare was nearly identical for the nonthinned slash and loblolly pine, but the number of residual loblolly pine galls exceeded that of slash pine by nearly 300 total galls per hectare. In the treated areas, however, the percentage of gall mortality in slash pine was more than double that of loblolly pine.

The mean estimated proportion of gall surface areas that produced aeciospores over the 5 years of observation are shown in Table 4. For both species there was a slightly greater percentage of gall surface area producing spores in the thinned than in the nonthinned areas. Also, loblolly pine stem galls produced aeciospores over larger gall surface areas than did galls of slash pine, regardless of treatment.

DISCUSSION

As expected, thinning meaningfully reduced the mean numbers of stem galls per hectare. Our primary interests were in mortality and sporulation of the galls that remained.

Stem gall mortality in slash pine over the 5 successive years was relatively constant in both the thinned and nonthinned areas, averaging about 27%. Stem gall mortality in the nonthinned portions of loblolly pine plantations was similar to that in slash pine, but the mortality in the thinned areas of loblolly pine was only 7%. The most reasonable explanations for the greater mortality in the thinned slash pine were more severe infections in slash pine, resulting in a greater proportion of stem circumference encircled by galls; a higher percentage of stem gall cankering (Tables 1 and 2); and a greater incidence of stem breakage concurrent with stem galls (Belanger et al. these proceedings). It is generally accepted that loblolly pine is more tolerant of fusiform rust than is slash pine (Goddard and Wells 1977, Powers et al. 1974). That conclusion is supported by the rust associated mortality (RAM) that occurred in the nonthinned portions of our study plantations. The 5-year cumulative mortality in slash pine was nearly 40% greater than in loblolly pine in both volume (m^3/ha) and numbers of trees per hectare (Belanger et al. these proceedings). The decrease in mean numbers of branch galls per hectare in loblolly pine over the observation periods did not show the differences between the thinned and nonthinned areas as was true for stem galls; i.e., the 5-year rate of decrease in the number of branch galls was quite similar for the thinned and nonthinned, with loblolly pine mean numbers per hectare being nearly equal in the fifth observation period.

Thinning did not alter the percentage of stem galls that produced aeciospores for either pine species. There were indications of a time-dependent decline in sporulation that was very obvious in slash pine and present at the fifth year for loblolly. The conclusion that sporulation was not increased by the treatment is further supported by the fact that the mean frequency of stem gall sporulation was slightly higher in the nonthinned areas for both species. However, the possibility of a minor stimulation of aeciospore production by stem galls following thinning is suggested by the somewhat greater mean surface area of galls that produced aeciospores in the thinned areas over the 5 years that specific galls were observed (Table 4).

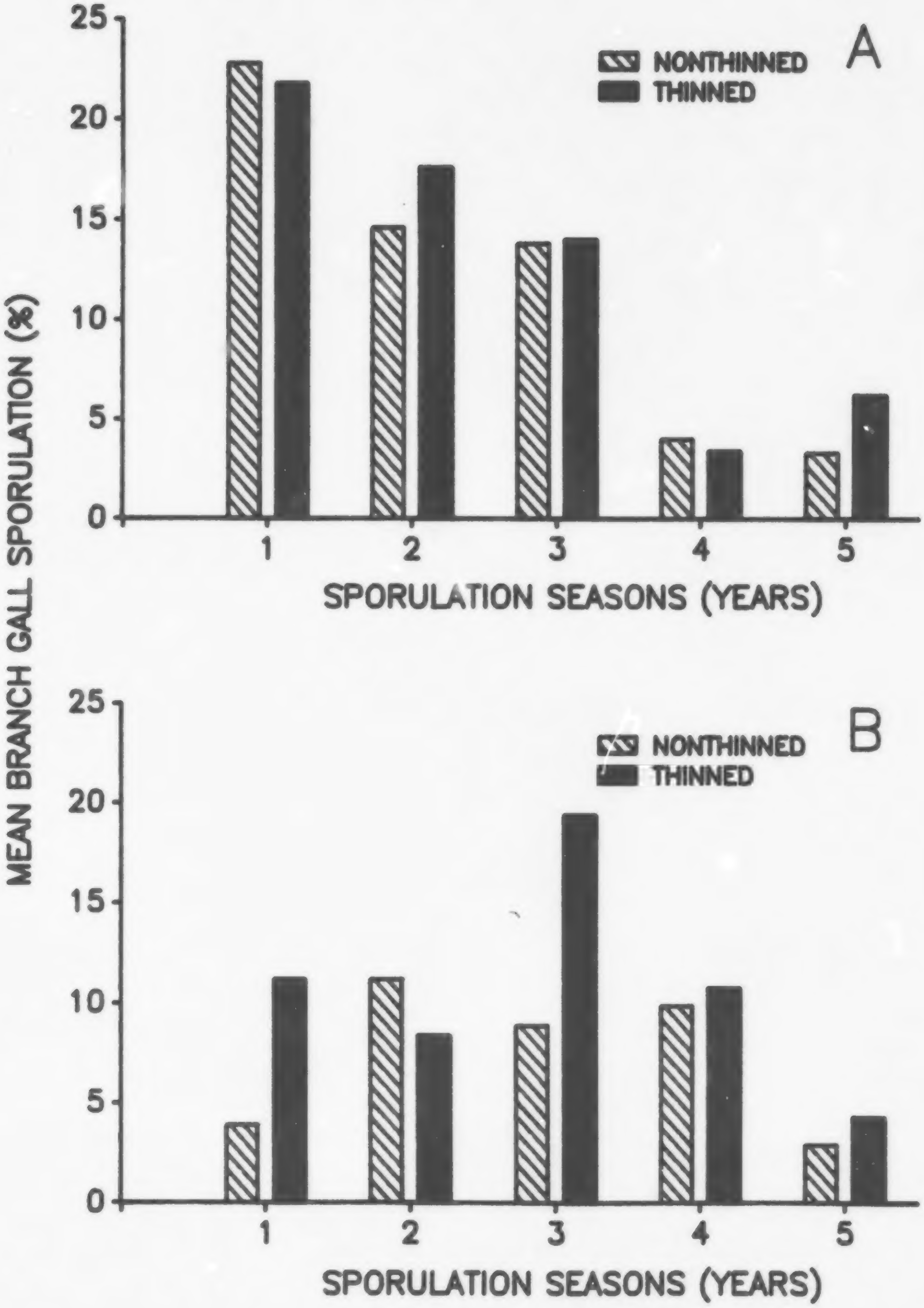

Figure 5. Mean percentage of branch galls of fusiform rust that produced aeciospores one through five years after thinning thinned and nonthinned portions of (A) 10 plantations of *Pinus elliottii* var. *elliottii* and **(B)** 11 plantations of *P. taeda*.

Table 3. Number of rust galls per hectare and percent mortality of total galls over five years in 10 *Pinus elliottii* var. *elliottii* and 11 *Pinus taeda* plantations

Observation period (years)	Living galls/ha			
	P. elliottii var. *elliottii*		*P. taeda*	
	Thinned	Nonthinned	Thinned	Nonthinned
1	509	1218	887	1715
2	363	1063	831	1432
3	313	909	762	1232
4	256	793	709	1093
5	224	697	680	987
Mortality (%)	56	43	23	42

Table 4. Estimated mean proportion of stem gall surface areas sporulating over five spore seasons (years)

Species	Mean gall surface area with aeciospores (%)			
	Nonthinned	Range 5 years	Thinned	Range 5 years
Pinus elliottii var. *elliottii*	21.2	20-22	24.6	18-28
Pinus taeda	28.9	22-36	33.2	28-42

A trend similar to the sporulation patterns of stem galls occurred in the branch galls of slash pine. With loblolly pine, however, the data show (Fig. 5B) large increases in the thinned areas in years 1 and 3 with smaller differences in years 4 and 5. These data must be interpreted cautiously because, unlike stem galls, the identity of individual branch galls could not be maintained. Therefore, the increase in sporulation in years 3 to 5 could be due either to a stimulation of sporulation of residual galls or sporulation of galls resulting from new infections since the thinning. This latter suggestion would not explain the difference in year 1, which might be explained more plausibly by a stimulation resulting from thinning.

In previous research on the dynamics of aeciospore production, Kuhlman (1981) recorded a mean stem and branch gall sporulation of about 55% and gall mortality of about 33%, over four spore seasons in two loblolly pine plantations aged 9 and 11 years at the start of the study. Chappelka and Schmidt (1983) observed aeciospore production in a slash pine plantation for 6 years, from age 10 to 16 years. They recorded a decrease in living branch and stem galls of 71% and a reduction in galls producing aeciospores from 73 to 23% over the seven seasons.

Our data from the nonthinned portions of the slash plantations, with an average plantation age of 16 years, show an initial mean sporulation (stem and branch galls) of 16%, which declined to 10%

after 5 years. Total numbers of living galls per hectare decreased by an average of 43% over the same 5-year period. These data indicate a general continuation of the trends in sporulation and gall mortality reported by Chappelka and Schmidt (1983) for slash pine between ages 10 and 16 years.

In the nonthinned loblolly pine areas, there was a definite decrease over 5 years in the numbers of both living stem and branch galls. For the percentage of loblolly pine galls sporulating, the data were too variable between years to indicate a definite decreasing sporulation trend; however, the recorded percentage sporulation for year 5 was less than for the previous four seasons.

Although there may have been a slight increase in the surface area of stem galls producing aeciospores, such a stimulatory effect would be dwarfed by the reduction in gall numbers resulting from thinning and natural mortality, especially death of branch galls. We observed no other stimulatory influence of thinning that could be interpreted as increasing aeciospore production.

At the end of our 5-year study, the majority of the fusiform rust galls in both slash and loblolly pine with the potential to produce aeciospores were stem galls located mainly in the lower 4.5 m of the stem (Figs. 1 and 2). The nonthinned areas contained an average of 395 and 334 more stem galls per hectare than did the thinned areas for slash and loblolly pines, respectively. These data, combined with the decrease over time in the numbers of branch galls in both species regardless of treatment, suggest that the removal of trees with stem galls in stands 16 years or older may significantly reduce potential inoculum available for spreading the disease to adjacent areas. How such a reduction would affect rust incidence, however, will need to be evaluated by research such as that of Schmidt et al. (1982), designed to quantitatively assess the practical value of sanitation-salvage thinnings on the spread of the fusiform rust disease from localized sources of aeciospore production.

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A NEW STRATEGY FOR THE BIOLOGICAL CONTROL OF PINE STEM RUSTS

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INTRODUCTION

As a group, pine stem rusts are considered the most important forest tree disease problem in Canada (Whitney et al. 1982). There is extensive documentation pointing out their significance in intensively managed Canadian pine forests (Baranyay and Stevenson 1964; Bella 1985a, 1985b; Carlson 1969; Hiratsuka 1981; Hiratsuka and Powell 1976; Hiratsuka et al. 1988; Johnstone 1981; Powell and Hiratsuka 1973; Ziller 1974). Development of effective, economically feasible, and environmentally acceptable control and management strategies of the pine stem rusts is important for successful cultivation of pines.

Although breeding for rust-resistant planting stock in conjunction with tree improvement programs may be the ultimate solution for controlling this group of diseases, there are many obstacles and unknown factors to be considered before any practical results can materialize from this approach. Chemical control of certain species in specific cultural conditions can be justified (Kistler and Merrill 1978; Merrill and Kistler 1976a, 1976b), but the results are often inconclusive (Leaphart 1963), economically unfeasible, and environmentally unacceptable in most forestry situations. Silvicultural controls such as alternate host eradication (Offord et al. 1958), and pruning of lower branches (Hunt 1982) have been suggested but results are not always clear.

Biological control can be considered as an alternate strategy for controlling this group of pine diseases. Several aggressive mycoparasites and other fungi and bacteria associated with pine stem rusts have been identified and investigated (Ayer et al. 1980; Bergdahl and French 1978; Byler and Cobb 1969; Byler et al. 1972a, 1972b; Hiratsuka et al. 1979; Kuhlman et al. 1976; Pickard et al.1983; Powell 1971b, 1971c, 1972a; Tsuneda and Hiratsuka 1979, 1980, 1981b; Tsuneda et al. 1980; Wicker and Wells 1968). Possible roles of these mycoparasites in the epidemiology of pine stem rusts and the possibility of their use in the biological control of those fungi have been suggested and discussed (Byler et al. 1972a; Hiratsuka 1979; Hiratsuka et al. 1987; Powell 1971e; Powell 1974; Quick and Lamoureux 1967; Tsuneda and Hiratsuka 1981a). One of the biggest obstacles of this approach is the difficulty of effectively delivering selected hyperparasites to the target organisms.

Insects and other free-moving organisms such as mites and slugs are known to feed on pine stem rust spores and rust-infected tissues. It is suspected that these organisms play a significant role in the epidemiology of the diseases caused by the rusts (Myren 1964; Nelson 1962, Powell 1971a; Powell 1971d; Powell 1972b; Powell 1974; Powell and Skaley 1975; Powell et al. 1972; Snell 1919; Wong 1972).

PROPOSAL FOR A NEW STRATEGY

This proposal is a new strategy for biological control of pine stem rusts with aggressive hyperparasites using certain free-moving organisms (mainly insects) as possible vectors of hyperparasitic microorganisms. If these vectors are species which actually seek the target organisms (pine stem rusts) and feed on the rust sori, we can potentially contaminate these vectors with active propagules of hyperparasites and release them into the areas with high rust populations.

CANDIDATE ORGANISMS

With this new strategy in mind, literature searches, field surveys, and laboratory examinations involving western gall rust were conducted in order to find suitable candidate vectors and mycoparasites.

Among the insects and other free-moving organisms recorded on western gall rust and other pine stem rusts by Nelson (1982), Powell (1971a), Powell and Skaley (1975), and Powell et al. (1972), several frequently identified species seem to feed selectively on pine stem rusts. They are *Mycodiplosis* spp. (Diptera: Cecidomyiidae), *Phalacropsis dispar* (LeConte) (Coleoptera: Phalacridae), and *Epuraea obliquus* Hatch (Coleoptera: Nitidulidae) (Fig. 1). At the present time, the most promising insect candidate is *E. obliquus*. This species and other nitidulids are known to feed on tree sap and associated fungi (Hatch 1952; Parsons 1967).

The best candidate mycoparasite is *Scytalidium uredinicola* Kuhlman, Carmichael and Miller. This is one of the several aggressive mycoparasites of western gall rust identified from previous work (Hiratsuka et al. 1979; Tsuneda and Hiratsuka 1979, 1980, 1981a, 1981b; Tsuneda et al. 1980). This fungus parasitizes immature spore layers as well as mature spores and is capable of destroying the entire spore crop for the year.

There are strong indications that *E. obliquus* is the main vector of *S. uredinicola* and other mycoparasites in nature. The beetle can carry spores of *S. uredinicola* on most of its body surfaces, especially on areas having setae (Figs. 2, 3).

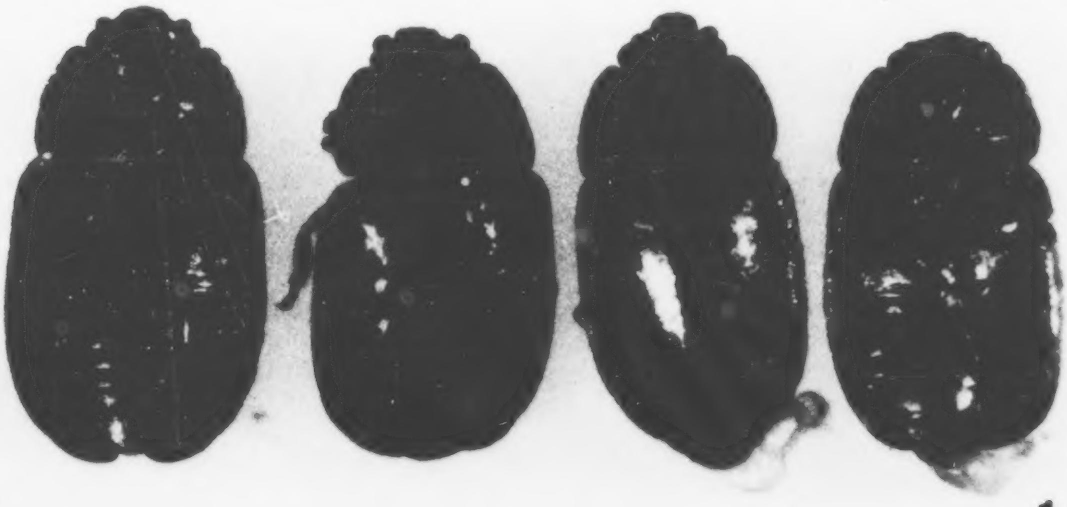

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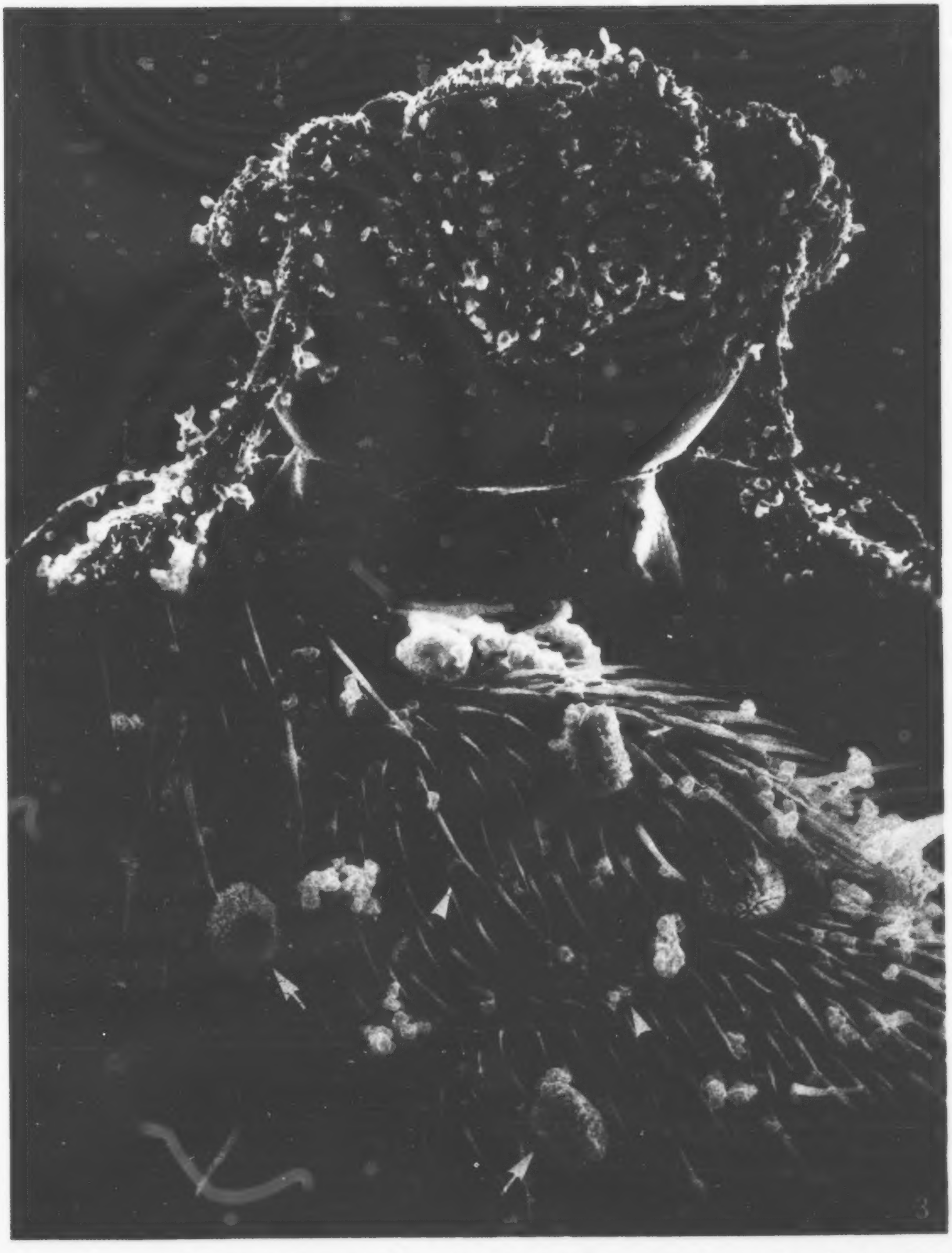
Figure 1. Adults of *Epuraea obliquus* (× 30).



Figures 2-3. 2. A head of *Epuraea obliquus* (× 90). 3. Spores of *Endocronartium harknessii* (large spores, arrows) and spores of *Scytalidium uredinicola* (smaller spores, arrowheads) on an antenna of *Epuraea obliquus* (× 400).

LIFE CYCLE OF *EPURAEA OBLIQUUS*

The life cycle of *E. obliquus*, as suggested by preliminary observations made in Hinton, Alberta, area in 1988 and 1989 is as follows. Overwintering adults emerge from the duff in early June to seek rust galls. After a few days of feeding on rust spores, the adults mate and lay eggs in the rust sori. The eggs hatch in a few days, and the larvae start feeding on rust spores. After 2-3 weeks, larvae mature (by the time most of the spores are consumed by the larvae) and drop to the ground. The larvae move under the duff layer just above the mineral soil where they pupate a few weeks later, and in another few weeks emerge as adults and remain in the duff where they then enter diapause.

CONCLUSIONS

This new biological control strategy for plant pathogens, using aggressive microbial hyperparasites with certain free-moving, target-seeking organisms (mainly insects) as vectors (Fig. 4), has much merit and can be applied to various pathogen-hyperparasite systems in forestry and agriculture. A joint investigation involving an entomologist (J. Volney, Northern Forestry Centre, Forestry Canada, Edmonton, Alberta), a natural product chemist (W.A. Ayer, Department of Chemistry, University of Alberta, Edmonton, Alberta), and a forest mycologist (Y. Hiratsuka, Northern Forestry Centre, Forestry Canada, Edmonton, Alberta) has been proposed to develop this idea further.

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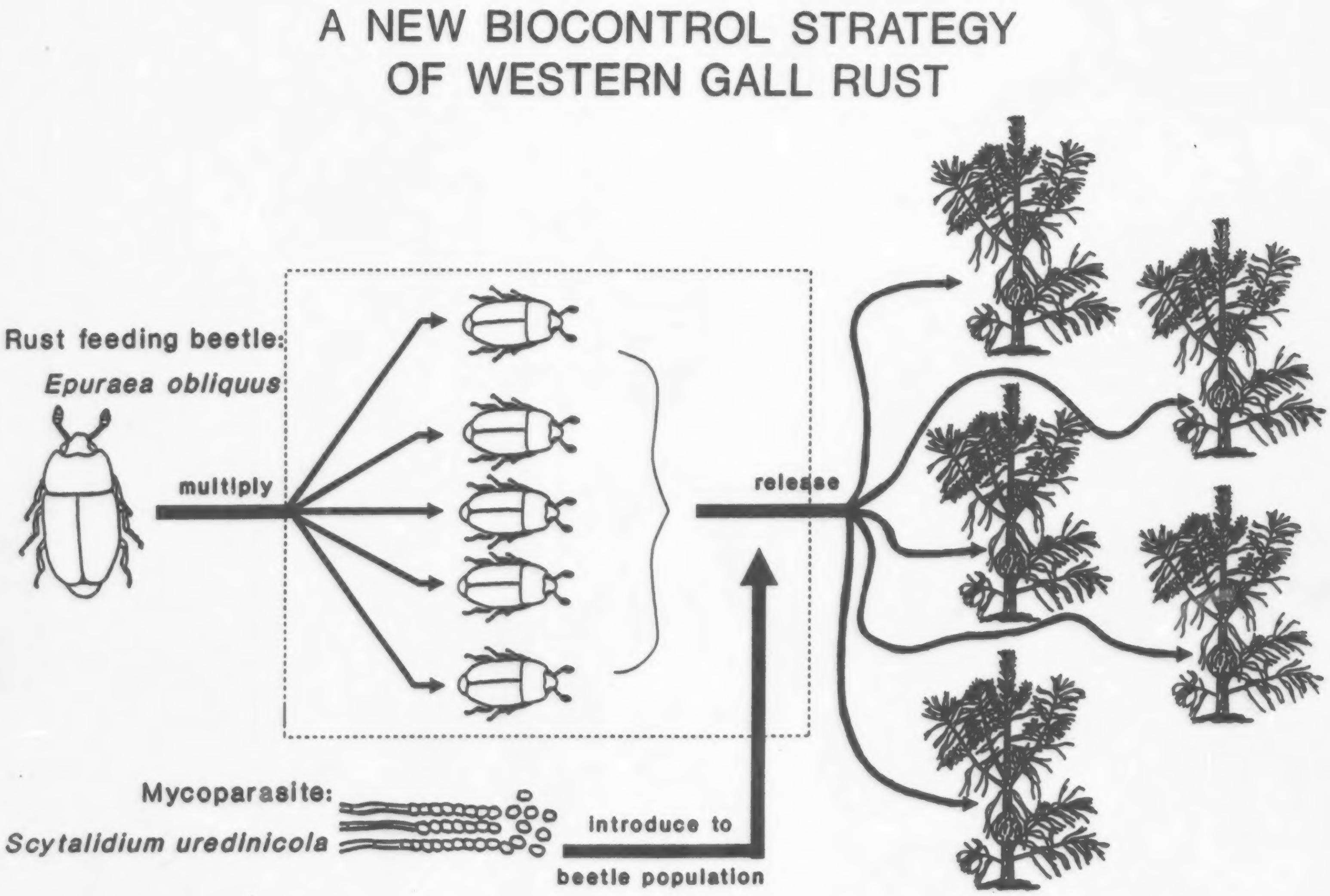
A NEW BIOCONTROL STRATEGY
OF WESTERN GALL RUST
Rust feeding beetle:
Epuraea obliquus
multiply
release
Mycoparasite:
Scytalidium uredinicola
introduce to
beetle population

Figure 4. A schematic drawing of the new biocontrol strategy of western gall rust.

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WHITE PINE BLISTER RUST CONTROL IN A 5-YEAR-OLD EASTERN WHITE PINE PLANTATION AT VERCHERES, QUEBEC (1984-88 RESULTS)

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ABSTRACT

Between 1984 and 1988, inclusively, the annual examination of 631 trees in a 5-year-old white pine plantation revealed that, without control, the level of trees affected by white pine blister rust would have risen from 11.7 to 19.2% . Yearly trimming of cankered branches during that period reduced the proportion of diseased or killed trees to 9.7%. Measurements of distance from the stem, and height above ground, of existing and new branch and stem cankers permitted elaboration on other control scenarios. For example, one systematic pruning of lower branches in 1984 would have yielded a 5.2% tree mortality by 1988, while yearly removal of branch and trunk cankers would have yielded a loss of 6.3%.

INTRODUCTION

In eastern Canada, white pine (*Pinus strobus* L.) plantations are often limited in area and usually located on privately owned lands. In such plantations, costs of several control scenarios can be accepted more easily than in larger plantations.

At Vercheres, Quebec, 5000 eastern white pine seedlings were planted in 1979 on a flat, imperfectly drained, sandy soil, to serve as rootstock of a seed orchard. In July 1984, a rapid survey for the presence of white pine blister rust (*Cronartium ribicola* J.C. Fisch.) indicated a level of infection of 11.7% of the trees, mostly branch-infected. Since those trees were part of another experimental design (grafting in 1983 or 1984), we proposed the detection and destruction of the alternate *Ribes* in the plantation site, and removal of infected branches, as immediate control action.

The study reported here was then initiated to compare various rust control actions in that 5-year-old plantation. For a 5-year period, a yearly follow-up of the rust infection level was realized in the whole plantation, but detailed observations were possible only on a block of 631 trees on which grafting action was not yet initiated at the beginning of our study in 1984.

METHODS

The plantation is located a few kilometres east of Montreal Island (alt. 30 m, lat. 45°41′00", long. 73°19′00") on 4.5 ha of sandy soil. Spacing between trees is 3 × 3 m. The heights and annual terminal growths could not be fully documented as in other plantations because of grafting actions already initiated in the major part of the plantation. However, tree height averaged 1 m in 1984.

The whole plantation was divided into four blocks of unequal size that were treated over different periods. In block 1 (631 trees), yearly removal of affected branches was performed from 1984 to 1988 inclusively (5 years), while block 3 (975 trees) was similarly treated 4 years (1985-88), and blocks 2 and 4 (1076 and 567 trees) were treated only 3 years (1986-88). These actions coupled with *Ribes* eradication in 1985 lowered the inoculum load.

At the time treatment was made, canker characteristics were measured. The following data were collected: height above ground; length of canker; diameter of branch or trunk affected (at base of canker); proportion of circumference affected; and, for branch cankers, distance of canker proximal margin from the trunk. Also, after 1984, if a trunk canker was noted, we determined if it originated from a previously trimmed cankered branch. In addition to providing the general level of branch and trunk infection, such measures were intended to indicate the progress of the rust from branches to trunks and the rate of mortality of infected parts in such a young plantation. Data from the four blocks were used when dealing with the proximal distance of branch cankers and the height above ground of trunk cankers, because they were comparable throughout the plantation.

The various treatment scenarios presented in the results were considered for block 1, the only one that permitted the analysis of 5 years' results. Yearly trimming of infected branches was the treatment performed and yielded direct results. Other figures were deducted from field data recorded yearly.

Infected living trees and those killed by the rust as observed in 1984 determined the initial infection level. In subsequent years, to establish the total infection level (without treatment), the percentage of trees newly affected or killed (after deduction of trunk infections resulting from unsuccessful previous branch trimmings) was added to the infection level of the previous year.

To calculate infection levels after the 1984 systematic pruning of lower branches, new trunk cankers (from infected branches unsuccessfully pruned) and new infections observed above 30 cm from the ground were added annually to the number of trunk cankers present in 1984.

Finally, in the scenario where branch and trunk cankers are removed yearly, the number of trees lost (killed or cut) was equivalent to the cumulative number of trunk cankers observed during the 5-year period (since healthy branches were left and served as infection courts the same way they did when only branch cankers were trimmed).

RESULTS

Early Symptoms

The detailed examination of lower branches revealed an important number of infections at the stage of branch or trunk swellings, and bark discoloration. Usually at that stage, the branch foliage is still green and the canker is often overlooked by untrained observers. This type of early symptom, when detected, can raise significantly the observed level of infections in very young plantations. When the affected branches are visibly dried, then anyone can record them. Spermogonia and aecia become evident to the observer.

Progress

In 1984, 11.7% (or 74) of the 631 trees examined in block 1 were affected by white pine blister rust (Fig. 1); of those, 67 showed only branch cankers. In 1988, without control action, 121 trees

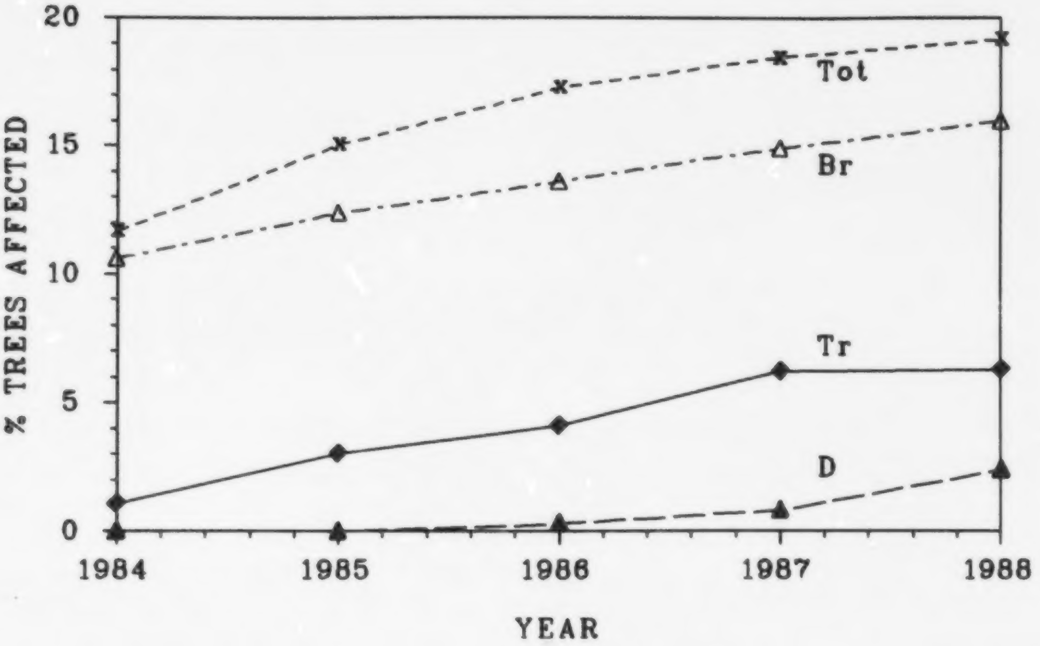

Figure 1. Evolution of the white pine blister rust in a 5-year-old eastern white pine plantation. The percentage of trees killed (D), affected to the trunk (Tr), affected to branches only (Br), and the total percentage of trees (Tot) affected are shown for the 5-year period (Block 1, 631 trees).

(19.2%) would have been attacked; one hundred trees (16%) affected on branches, and at least 55 trunks cankered or killed. If such an infection rate (1.5% per year) were maintained, more than 50% of the trees would be affected before the plantation reached the age of 50. The proximal margins of all branch cankers (except 2) were less than 20 cm from the trunk and would have reached that trunk within the next 2 or 3 years. Trees killed or trunks affected by 1988 would have been even greater in number if inoculum load had not been reduced by *Ribes* eradication in 1985 and by yearly trimming of affected branches in the whole plantation. Therefore, the reported total level of infection represents a minimum that could have been higher under undisturbed conditions.

Treatments

Annual trimming of affected branches helped to maintain the number of fatally affected trees at 61 (9.7%) in 1988, including trees killed by trunk cankers that were not destroyed in 1984 because of grafting experiments (Fig. 2). Such a sanitation-trimming yielded reductions in the amount of fatal attacks in the order of 69, 67, 56, and 49%, respectively, for each of the 4 years after the beginning of the observations (general level of infection minus percent affected when trimmed yearly). Instead of this treatment, if a single systematic pruning of branches lower than 30 cm above ground level had been made in 1984, we would have had 33 cankers (5.2%) on the trunks affected or killed in 1988. Similar results

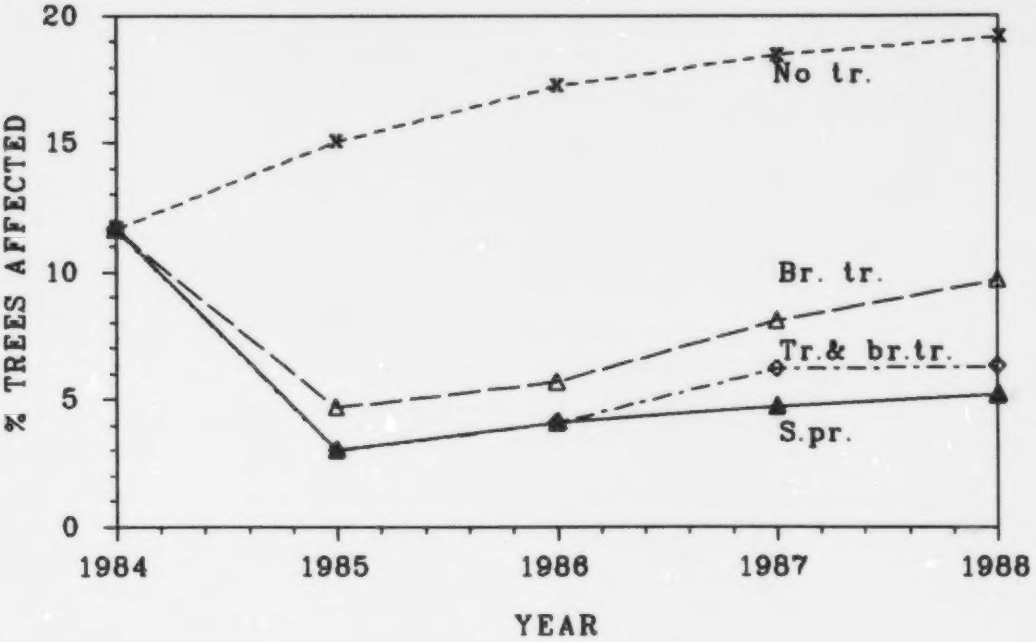

Figure 2. Levels of white pine blister rust infection observed (No tr.) and effect of annual trimming of affected branches (Br. tr.), annual removal of affected trunks and branches (Tr. & br. tr.), or systematic pruning (S. pr.) of the lower branches in 1984 only (Block 1, 631 trees).

were obtained over a 9-year period by Lehrer (1982) in Wisconsin. On the other hand, the annual destruction of trunk and branch cankers from 1984 to 1988, inclusively, would have resulted in a loss of 40 trees (6.3%) in 1988, as compared to 19.2% without control.

Among other scenarios (not illustrated), the destruction of affected trunks and branches in only 1984 would have yielded 8.9% of the trees affected in 1988; two successive trimmings (1984 and 1985) would have yielded 7.6% of the trees affected (Table 1).

The time needed for examination, data-recording, and branch-trimming was about 1 hour for 80 to 90 plants, with a team of two persons (about 0.8 ha/day). With the same team, it might take longer to prune lower branches (0.5 ha/day), but over a period of 5 years it is less expensive (since it is needed only once) and the control should be better. The time needed to perform other control measures listed in Table 1 would be intermediate between these two extremes.

Canker Characteristics on Branches and Trunks

In the whole plantation (four blocks), the majority (70 to 80%) of branch cankers were less than 10 cm from the trunk in 1984, 1985, 1986, and 1987 (Fig. 3). In 1988, when the plantation reached age 10, the number of new branch cankers was reduced (15) because we trimmed annually, but by then,

Table 1. Results in 1988 of various control scenarios against white pine blister rust at Vercheres, Quebec (Block 1, 631 trees)

Treatments	Trees affected (%)
1. No treatment	19.2
2. Annual trimming of affected branches (1984-88 inclusive)	9 7
3. Removal of affected trunks and branches (1984 only)	8.9
4. Removal of affected trunks and branches (1984 and 1985)	7.6
5. Annual removal of affected trunks and branches (1984-88 inclusive)	6.3
6. Systematic pruning 30 cm above ground (1984 only)	5.2

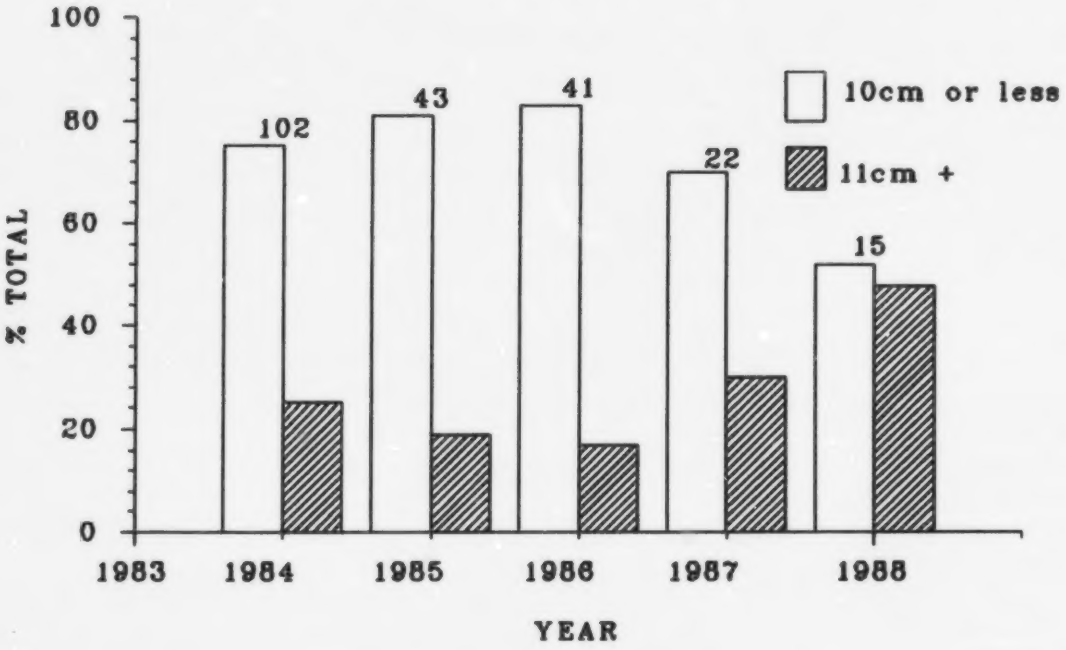

Figure 3. Distance of the proximal margin of branch cankers to the trunk. The total number of branch cankers observed each year is shown above the bars.

50% of the proximal margins of the cankers were located within 10 cm and 50% were further than 11 cm from the trunk. Infections proceed from the needles; therefore, new cankers on lower branches will allow more time for subsequent prunings or trimmings.

Trimming branch cankers when their proximal margin is less than 10 cm from the trunk succeeded in 30 to 40% of the cases, but in the remaining cases trunk infections could not be avoided. This result endorses the 12.5-cm limit proposed by King (1958) for successful branch-pruning in young plantations. Usually, such branch cankers reach the trunk one year after they are observed, and kill the 6- to 9-year-old tree within 3 years after reaching the trunk.

Favorable conditions for rust infection frequently occur near ground level (Van Arsdel et al. 1956). In the whole plantation, between 87 and 96% of the trunk cankers were located 30 cm or less from the ground during the period under observation. This reinforces the fact that an early systematic pruning of lower branches in young plantations, particularly when herbaceous vegetation is abundant, may be needed to ensure good control against white pine blister rust. In a number of plantations in Quebec, eastern white pine has a propensity to lower-branch proliferation. Trees should be carefully examined because cankers on the branches automatically mean the death of the tree; and, in some cases, it might mean the difference between a successful plantation or a failure within the first 10 to 15 years.

CONCLUSIONS

From these results, a number of conclusions can be drawn.

1. A detection survey when the plantation is 5 years old is important for determining the need for early control.

2. A systematic pruning of lower branches (30 to 40 cm above ground) and destruction of affected trunks would be really beneficial in some 5-, 6-, or 7-year-old plantations of Quebec if white pine blister rust reaches a significant level of infection (8 to 10%) at that age. In the present study, very few trunk infections were located over 30 cm from the ground during that 5-year period.

3. With an original level of infection of over 10% of the trees, the destruction of affected trunks and branches for two consecutive years can also be prescribed to reduce significantly the number of fatal attacks over the next 5 years, but then trees are still exposed to new infections at the bases of their trunks. A better control seems possible by the removal of one or two whorls every 2 years (Weber 1964; Lehrer 1982).

Additional studies are under way in other locations of Quebec, that should permit us to determine if further control actions are needed in aging plantations in various hazard zones (Lavallée 1986). Our results confirm those for *Pinus monticola* Dougl. in British Columbia (Hunt 1982) and eastern white pine in Minnesota (Stewart 1957), which demonstrate that branch removal as early as possible will improve white pine survival and that two prunings might be needed before harvesting plantation trees when control measures must be applied so early because of the infection level.

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INTEGRATED CONTROL OF BLISTER RUST IN A KOREAN PINE PLANTATION IN HEILONGJIANG PROVINCE, THE PEOPLE'S REPUBLIC OF CHINA

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ABSTRACT

This study dealt with integrated control of blister rust (*Cronartium ribicola* J.C. Fisch.) on *Pinus koraiensis* Sieb. et Zucc. in a 22-year-old plantation in Heilongjiang province. Integrated control was carried out by using pruning, thinning, and fungicides according to disease severity, growth, and treatment cost and was based on a study of regularities of occurrence and development of this disease. Pruning to a height of 1.5 m, thinning to levels of 20-30% (residual stocks 1700 stems per hectare), smearing pine tar and diesel oil (1:1 by volume) on infected trees with disease class lower than 3, and controlling disease incidence lower than 4% resulted in greater tree growth in both volume and diameter, fewer losses from disease, and lower costs.

INTRODUCTION

Korean pine (*Pinus koraiensis* Sieb. et Zucc.) is one of the most commercially valuable species in northeastern China. This five-needle pine is native to the area. Blister rust (*Cronartium ribicola* J.C. Fisch.) which was discovered about 40 years ago (Cummins and Ling 1950) severely affects this pine throughout its range in northeast China (Shao et al. 1980). The disease is particularly severe in plantations. Since 1978, researchers have studied and attempted to control blister rust. Blister rust inoculum is reduced within stands by cutting or using herbicides against the alternate hosts, species of *Pedicularis* and *Ribes*, and also by felling severely damaged trees (Research Group on Blister Rust 1979; Ju et al. 1979). Research has demonstrated that pine tar brushed onto cankers with vertical scribe cuts is effective in blister rust control (Ju et al. 1979). The Research Group and Ju et al. recommended pruning to control blister rust, and Guo and Chuei (1988) carried out field experiments that showed the effectiveness of pruning. However, when these practices were used by foresters, it was found that losses from the disease continued to increase for 2 or 3 years after treatment (General Forest Bureau of Heilongjiang 1981). Thus, additional studies were needed to determine the conditions under which such practices are worthwhile for managing the disease.

The objectives of this study were to determine 1) the occurrence and development of blister rust in a 20-year-old Korean pine plantation, 2) the best season and time of disease development for applying fungicides, 3) methods for reducing disease spread after pruning and thinning, and 4) if integrated methods could be developed for more economical control of blister rust.

MATERIALS AND METHODS

Disease Occurrence and Spread on Stems

The survey was made in a 22-year-old Korean pine plantation at the Hua-Yang Forest Farm, Heilongjiang province. The plantation had a western exposure on a 10-25° slope, and the trees averaged 8 cm in diameter at breast height (dbh) and 5.5 m in height. Two adjacent rows of seven contiguous plots (0.2 ha each) were established from the lowest to highest portions of the plantation, and eight other midslope plots were established; i.e., there were 22 plots. Tree growth, incidence of stem cankers, and disease classes were recorded twice each year during the test. For statistical analysis, correlations were made between dbh and disease severity, increase in numbers of infected trees, tree mortality, and initial disease incidence. Annual growth was determined by measuring the width of annual rings in these sections. In this study, disease was divided into five classes (Table 1), which differ from the ratings used by the Research Group (1979).

Control Methods

Pruning

The trees in four plots with similar stand density and plot slope were pruned (all dead and living branches) to heights of 1.5, 1.8, and 2.1 m.

Thinning

Stems per hectare were reduced by 23-48% in six plots (Institute of Forest Soil 1982). The stand factors before and after spacing (precommercial thinning) are shown in Table 2.

Fungicides

Four fungicide treatments were applied to rust cankers: pine tar, pine tar with diesel oil (1:1 by volume), triadimefon, and triadimefon with diesel oil. The treatments were applied to cankers with and without vertical scribe cuts. Treatment times were 1) spring (aecia period), 2) fall (spermogonia period), 3) fall and spring, and 4) two successive springs. The treatments were applied to individual trees in the stand adjacent to the check plots.

Table 1. Disease classes of Korean pine blister rust

Class	Characteristics
0	Not infected
1	Branch infected or main stem infected, canker width less than 1/3 of stem
2	Stem infected, canker width from 1/3 to 2/3 of stem
3	Canker width 2/3 to girdled, vertical length <40 cm
4	Canker girdled, vertical length >40 cm, tree dying or killed by rust

Table 2. Stand factors before and after thinning for blister rust control

Plot no.	Level of thinning (%)	Before thinning				After thinning			
		Density (stems/ha)	Mean dbh (cm)	Volume (m^3/ha)	Blister rust incidence (%)	Density (stems/ha)	Mean dbh (cm)	Volume (m^3/ha)	Blister rust incidence (%)
10	23.0	2012	6.5	33.9	6.1	1556	7.5	26.5	6.3
8	29.0	2354	7.3	38.2	0.9	1681	7.6	28.6	0.6
9	42.0	3212	7.2	45.5	0.6	1809	7.5	27.8	0.6
7	Check	3059	8.0	46.9	1.8	3059	8.0	46.9	1.8
12	Check	2537	8.6	47.9	6.8	2537	8.6	47.9	6.8
15	48.0	2895	7.0	47.9	9.6	1505	8.2	30.2	5.0
14	31.5	2240	8.9	53.8	5.0	1535	9.3	40.6	1.3
21	26.3	2130	8.3	41.9	6.2	1570	8.5	33.3	3.7
20	Check	2520	7.8	45.8	7.1	2520	7.8	45.8	7.1

Integrated control

In 1986, on a 10-ha plot within the stand, trees were thinned (20% level) and the branches pruned up to 1.5 m. Dead, dying, and severely rust-infected trees were removed. Stem cankers on the remaining trees were treated with pine tar with diesel oil as before. Records were also kept for the costs of all treatments, work time, and incremental value of tree volume.

RESULTS

Disease Occurrence and Development

Stand Density and Incidence of Diseased Trees

Data were gathered from 16 plots. More infected trees were observed in the plots with either many or few stems per hectare. The plots with 1700 stems per hectare were lightly affected (Table 3).

Dbh and Diseased Tree Incidence

More than 60% of the diseased trees were in the most severe disease class, class 4; and both incidence of diseased trees and disease severity were inversely related to diameter class (Table 4).

Stem Canker Origin and Height

Most stem cankers occurred at a height of 0.1-0.8 m, and they were especially prevalent between 0.25 and 0.35 m (Fig. 1). Stem infection originated from infections at branch axils or from branch infections that spread to the main stem.

Incremental Incidence and Mortality of Infected Trees

Surveys from 1984 to 1988 showed the percentage of infected trees and mortality increased each year depending on the initial rate of infection. The linear regression correlation for these relationships were significant at the 1% level (Fig. 2). The regression equations were

$$\hat{Y} = -0.14761 + 1.4417X$$

where Y = % of infected trees, and X = initial rate of infection.

$$\hat{Y} = 0.05447 + 0.9306X$$

where Y = rate of mortality, and X = initial rate of infection.

Table 3. Relationship of stand density and incidence of blister rust diseased trees

No. of plots	Density (stems/ha)	Dbh (cm)	Height (m)	Diseased trees (%)
2	1279	8.9	6.0	13.7
3	1642	8.5	5.5	2.4
6	2152	8.4	5.6	6.3
2	2529	8.2	5.5	6.7
3	2895	7.6	5.2	9.6

Table 4. Number of infected trees in each of four disease classes

Disease class	Dbh (cm)					Total no. of trees	Diseased trees (%)
	<6	8	10	12	14		
1	0	1	7	3	2	13	4.9
2	6	6	20	15	7	54	20.1
3	5	12	9	5	0	31	11.6
4	72	37	46	15	0	170	63.4
Total	83	56	82	38	9	268	
Total diseased trees (%)	31.0	20.9	30.6	14.3	3.4		100
Severity rating[a]	94.7	87.9	78.7	57.9	44.4		

[a] Severity rating = $\frac{\Sigma \text{ (All trees in the disease class} \times \text{class, i.e., 1 to 4)}}{\text{Total trees checked} \times \text{the highest class}}$

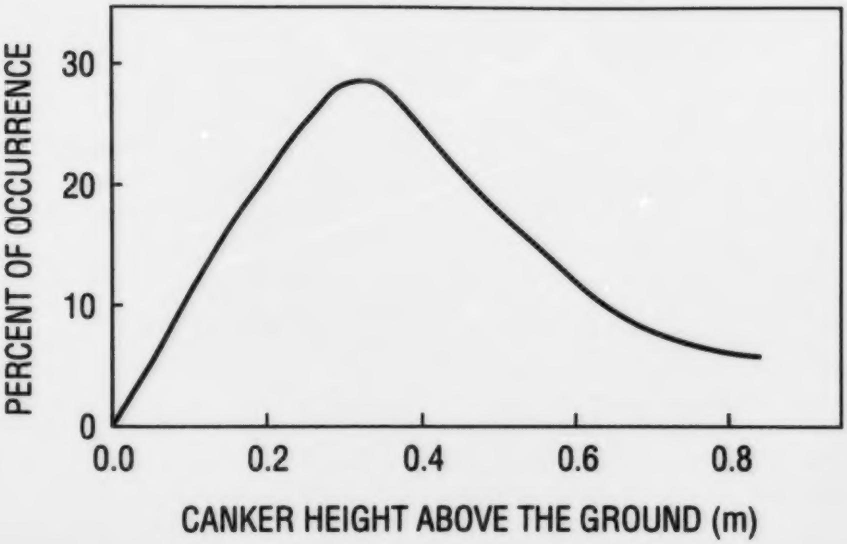

Figure 1. Incidence of stem cankers at various heights above the ground.

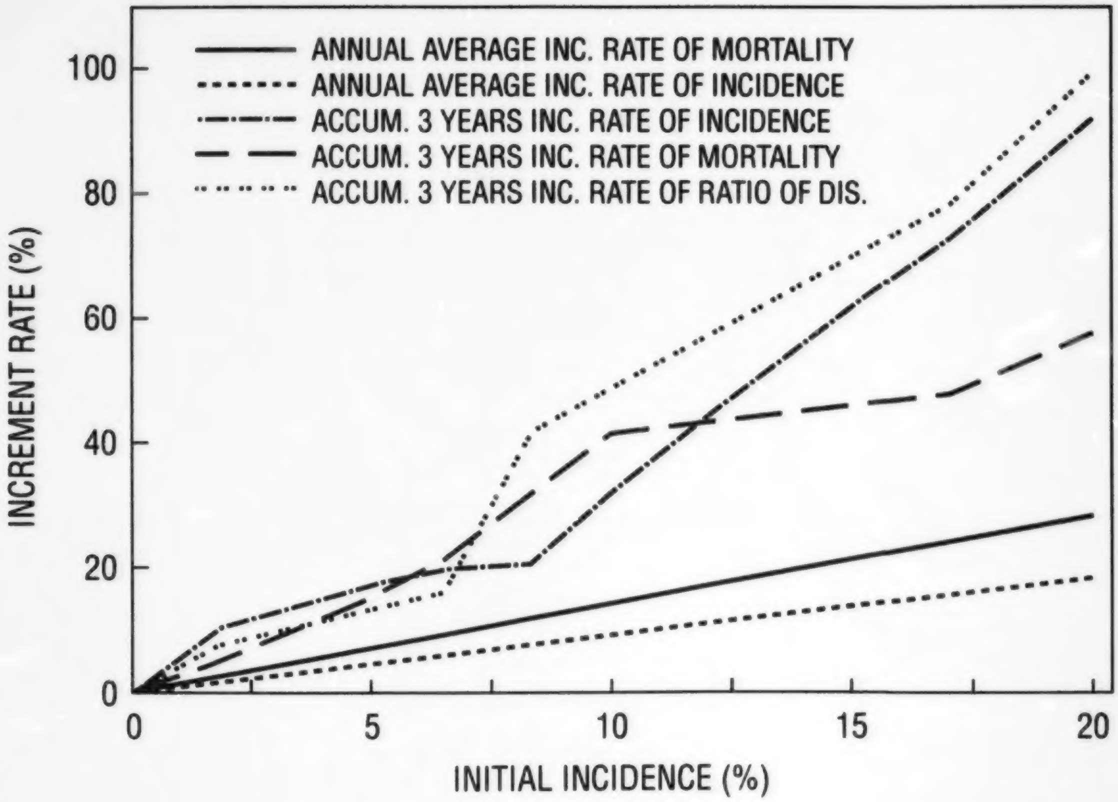

Figure 2. Correlation of increment of mortality and incidence with the initial incidence in plots.

Treatment Efficacy

Pruning and Thinning

After three years, the 1.5-m pruning resulted in the best disease control and tree growth (Table 5). Growth of each tree, stem canker incidence, and disease severity 3 years after different levels of thinning are shown in Table 6. At thinning rates of 23-48%, both stem diameter and stand volume increased. Compared with check plots, an increase in incremental diameter growth of about 95% occurred at 30% thinning, i.e., where about 1700 stems per hectare were left (plot 8). Plots 10, 9, 15, and 21 also showed better disease control, tree diameter, and volume growth.

Chemical Control

Effect of Different Chemicals Under the Same Treatment Conditions

Triadimefon, triadimefon with diesel oil, and pine tar all gave poor disease control, while pine tar with diesel oil gave satisfactory control (Table 7).

Table 5. Effect of pruning for blister rust control

Plot no.	Pruning height (m)	Incidence % Before treatment	 3 years after treatment	 Treatment response (%)	RCE[a] (%)	Mean dbh Before treatment	 3 years after treatment	 Treatment response (%)
11	1.5	10.5	11.4	8.6	74.6	8.8	10.8	22.7
6	1.8	6.4	7.6	18.8	44.7	8.2	10.0	22.0
5	2.1	11.9	13.9	16.8	50.3	9.0	10.9	21.1
12	Check	6.8	9.1	33.8	--	8.4	10.1	20.2

[a] Relative control effect = $\frac{\text{Severity weight in control} - \text{severity weight in treatment}}{\text{Severity weight in control}} \times 100$

Effect of Pretreatment Scribe Cuts on Fungicide Efficacy

When pine tar without diesel oil was applied to scraped cankers, disease control was 70% greater than with smearing alone; pine tar with diesel oil and smearing with scraping gave better results than just smearing (Table 7).

Effect of Treatment Time

Treatments applied in the fall (spermogonia period) were better than spring applications (aecia period), while applying the treatments in the fall and again the following spring was better than two successive spring applications (Table 8).

Effect of Chemicals Applied to Trees of Different Disease Classes

The rate of recovery of infected trees decreased as disease class increased, and there was no recovery of trees in classes higher than class 3 (Table 9).

Effectiveness of Integrated Controls

Effect of Treatment

The results were evaluated in June and September, 1987 (Table 10). Incidence of stem cankers was reduced to under 4.2% per hectare while other parameters were disease severity rating of 0.9, recovery rate of 53.5%, and control effectiveness of 80% in treated stands.

Table 6. Effect of thinning for blister rust control

Plot no.	Year of thinning					3 years after thinning				RDG[a] %		RVG[b] %		Incidence (%)	Severity rating (%)
	Density (stems/ha)	Dbh (cm)	Volume (m^3/ha)	IV[c] (%)	SR[d]	Dbh (cm)	Volume (m^3/ha)	IV (%)	SR	IAY[e] (%)	CAC[f]	IAY (%)	CAC[f]		
10	1556	7.5	26.5	6.3	3.8	9.6	52.5	7.0	5.4	9.3	60.3	32.8	74.8	11.1	42.1
8	1681	7.6	28.6	0.6	0.4	10.2	57.5	0.6	0.6	11.4	95.9	33.6	78.8	0.0	50.0
9	1809	7.5	27.8	0.6	0.5	9.7	57.1	1.6	1.0	9.8	68.0	35.1	86.7	166.6	100.0
7	3059	8.0	56.3	1.8	1.3	9.4	91.8	2.4	1.8	5.8	--	21.0	--	33.3	38.5
12	2520	8.6	56.4	6.8	4.1	10.1	86.4	9.1	6.5	5.8	--	16.5	--	33.8	58.5
15	1505	8.3	30.2	5.0	2.6	10.9	60.9	5.7	2.5	11.0	83.6	34.0	85.6	14.0	–3.8
14	1535	9.3	40.8	1.3	0.8	11.6	72.3	2.6	1.4	8.2	37.8	26.0	42.0	100.0	75.0
21	1570	8.5	33.3	3.7	1.9	10.7	65.5	2.6	1.2	8.6	44.3	32.4	76.3	–29.7	–36.8
20	2520	7.8	45.8	7.1	4.5	9.2	70.9	8.9	7.6	6.0	--	18.3	--	25.4	68.8

[a] Rate of diameter growth.
[b] Rate of volume growth.
[c] Incidence.
[d] Severity rating.
[e] Increment of annual average year.
[f] Comparison with control plots 7, 12, and 20.

Table 7. Effect of different chemicals and treatments on control of blister rust on Korean pine

Chemicals	Treatments	No. trees sampled	Severity rating Before control	 After control	AC[a] (%)
Triadimefon + 100×	Smearing	31	34.5	72.7	–5.8
Triadimefon + diesel oil (100:250)	Smearing	32	50.0	43.1	42.7
Pine tar	Smearing	31	52.7	54.5	30.0
Pine tar + diesel oil (1:1)	Smearing	33	63.1	18.5	104.1
Check	--	30	50.0	66.7	--
Pine tar	Scraping + smearing	28	60.4	18.8	84.7
Pine tar	Smearing	31	52.7	54.5	14.0
Pine tar + diesel oil (1:1)	Scraping + smearing	30	61.7	22.2	79.7
Pine tar + diesel oil (1:1)	Smearing	33	45.5	22.2	66.9
Check	--	29	65.2	75.8	--

[a] Actual control relative to the check.

Table 8. Effect of smearing pine tar for blister rust control in different seasons

Treatment season	No. stems	No. of times treated	Severity rating[a] Before control	 After control	AC[b] (%)
Late May 1986	31	1	55.5	53.6	19.7
May 1985 and 1986	36	2	58.8	33.8	58.8
Late Aug. 1986	31	1	45.5	22.2	67.5
Late Aug. 1986 and May 1987	29	2	53.3	0.10	116.1
Check	29	0	65.2	75.8	--

[a] See Table 4.

[b] Actual control relative to the check.

Table 9. Control of blister rust based on disease severity class

Disease severity class	No. stems	Severity rating		RCE[a] (%)	Rate of recovery[b] (%)	DSR[c] (%)	Mortality		RCE (%)
		Treatment	Control				Treatment (%)	Control (%)	
1	36	16.7	22.2	24.7	66.7	66.7	0.0	0.0	--
2	38	16.7	50.0	66.6	44.4	16.7	0.0	0.0	--
3	36	27.9	69.2	60.8	37.5	60.8	0.0	7.7	100.0
4	35	83.3	100.0	16.7	0.0	37.0	50.0	100.0	50.0

[a] Relative control effect = $\frac{\text{Severity weight in control} - \text{severity weight in treatment}}{\text{Severity weight in control}} \times 100$

[b] Rate of Recovery = $\frac{\text{Recovery stems}}{\text{Total sampled tree in treatment}} \times 100$

[c] Decline rate of stem severity rating.

Table 10. Integrated control of blister rust stem cankers on Korean pine

Plot	Area (ha)	Treatment	Blister rust incidence		Severity rating		RCE[a] (%)	AC[b] (%)
			Before	After	Before	After		
Treated	10.0	Thinned and pruned in 1986, smeared with pine tar + diesel oil in September 1986 and May 1987	16.9	4.2	8.8	0.9	87.1	97.4
Check	0.7	Smeared with pine tar in May 1986	9.4	11.4	6.9	7.3		

[a] Relative control effect.

[b] Actual control relative to the check.

Cost of Integrated Control

These costs were obtained from the individual tests by calculating hours of work time, amount of chemical used, and so forth (Table 11). The expenses of thinning, pruning, and applying (by smearing) fungicides were 15.00, 12.50, and 14.44 renminbi (RMB) per hectare, respectively, or a total cost of 41.94 RMB per hectare. (RMB is the recognized currency of the People's Republic of China; one RMB was equivalent to 0.34 Canadian dollars when this paper was prepared.) Korean pine stands can be protected by one control lasting 3 years or, as an added safeguard, an additional chemical control can be carried out within 5 years. If this is done, the total expense after 5 years is 56.38 RMB per hectare, or an average annual cost of 11.27 RMB per hectare.

DISCUSSION AND CONCLUSIONS

This study has shown that a density of 1700 stems per hectare in a 20-year-old Korean pine stand is beneficial for minimizing blister rust and stimulating tree growth. If there are more than 1800 stems per hectare in an infected stand, thinning should be carried out. Most stem cankers occur less than 1 m above the ground, and the majority of infections grow from branches to the main stem. Therefore, pruning can reduce both the amount of inoculum and the disease spread into stems. Pruning to 1.5 m is an effective control. The equations of increment rate of infected trees and mortality can predict disease severity of a stand 3-5 years in the future, based on the present incidence of diseased trees. Use of pine tar with diesel oil smeared on stem cankers (when disease class is lower than 3 in the autumn or the following spring) effectively controls blister rust.

Integrated management is not simply combining two or three kinds of controls. In this study, the economical results of integrated controls (pruning, thinning, and smearing chemicals) were based on our previous individual test results, as well as considering 4% incidence of stem cankers to be an economic threshold (Zhong et al. 1989). This study is limited to a 20-year-old plantation, an age when most damage from blister rust occurs (Ziller 1974; Boyce 1961; Shao et al. 1980). To gain more information about infection with aging, stands of various ages should be studied.

Table 11. Cost of blister rust control

Procedure	Stems treated per day	No. workers /ha	Amount of chemical (kg/ha)	Cost of chemical (RMB)[a]	Wages/day[b] (RMB)	Total (RMB)
Thinning	80-100	6	--	--	2.5	15.00
Pruning	350	5	--	--	2.5	12.50
Smearing	40	2	2 pine tar + 2 diesel oil	9.44	2.5	14.44
Cost over 3 years	--	--	--	--	--	41.94
Cost over 5 years	--	--	--	--	--	56.38

[a] RMB = renminbi.

[b] 1987 wage rate for casual worker.

Eradication of alternate hosts is an important strategy in blister rust control. Some reports suggested that there should be no alternate host plants within the edge of the stand, or that population should be controlled below 25 plants per hectare (Ziller 1974; Ju et al. 1979; Research Group 1979). However, a thorough search for 1 km around our plots, and observations about 50 km beyond the plots, failed to detect species of *Ribes*, *Pedicularis*, or other possible alternate hosts. In spite of this, newly infected trees appeared each year. Therefore, in canopied stands, the existence and population size of alternate hosts on the occurrence and development of blister rust will be reevaluated.

Application of pine tar has produced undesirable side effects on trees (Ju et al. 1979), but no such effects were observed in our study. In this study, scraping and smearing stem cankers with pine tar and diesel oil was slightly more effective than smearing cankers with some chemicals. Considering the extra cost of the scraping treatment, we preferred to just smear cankers with pine tar and diesel oil in the fall for integrated control. The simplicity of this technique is one of its major benefits. The cost of treatment as it relates to increased tree growth and control efficacy needs more study, especially at other localities, so that the economic guidelines can be further developed.

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WHITE PINE BLISTER RUST IN QUEBEC: PAST, PRESENT, AND FUTURE

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ABSTRACT

Eastern white pine (*Pinus strobus* L.) has not occupied the position it deserves in reforestation programs in Quebec during the past 25 years. Blister rust (*Cronartium ribicola* J.C. Fisch.) is the main reason for this aversion. A reevaluation of the incidence of white pine blister rust in Quebec was done in 1974 that proposed the subdivision of the white pine range into four susceptibility zones, two of which have a high potential for the production of this valuable species. Interest in *P. strobus* was then revitalized; a breeding program aimed at the development of new varieties with improved growth, form, and disease and insect resistance was initiated in 1977. The present status of the eastern white pine combined genetic research and breeding program is given in this paper, along with the research to be carried out in the future at the Laurentian Forestry Centre, Forestry Canada, Quebec.

HISTORY OF WHITE PINE BLISTER RUST

White pine blister rust was first discovered in Quebec in 1916 in the Montreal area by W.P. Fraser, who identified the fungus *Cronartium ribicola* J.C. Fisch. on leaves of *Ribes* at MacDonald College. Two years later, the pathogen was observed on eastern white pine (*Pinus strobus* L.) at the Seigniory of Perthuis in Portneuf County. The disease is now present over the whole range of eastern white pine in Quebec and North America. G.C. Piché (1917), founder and head of the provincial forest service, pointed out the danger of this introduced disease; he recommended temporarily suspending planting of this species in the Quebec area and proceeding with the complete eradication of *Ribes* bushes near nurseries and eastern white pine stands. At that time, eastern white pine was a major forest species on 96 000 km^2 in southern Quebec (Piché 1917).

In 1932, Pomerleau also pointed out the importance of the disease and recommended the complete eradication of the *Ribes* species in southern Quebec. In the 1930s, a study was conducted by Pomerleau (1932) to evaluate the importance of natural populations of eastern white pine in central Quebec and the extent of damages caused by the disease. In the area studied, eastern white pine covered 31 565 ha and, despite the fact that white pine blister rust was present over the whole area, only 3.5% of the trees in natural stands were infected.

Plantations were more severely attacked by the fungus. In many cases, over 50% of the young eastern white pines were infected, and rare were the examples where the plantations escaped the blister rust infection. It is not surprising, therefore, that most foresters of that time were reluctant to use eastern white pine for reforestation. In fact, no one would have recommended the species without *Ribes* eradication.

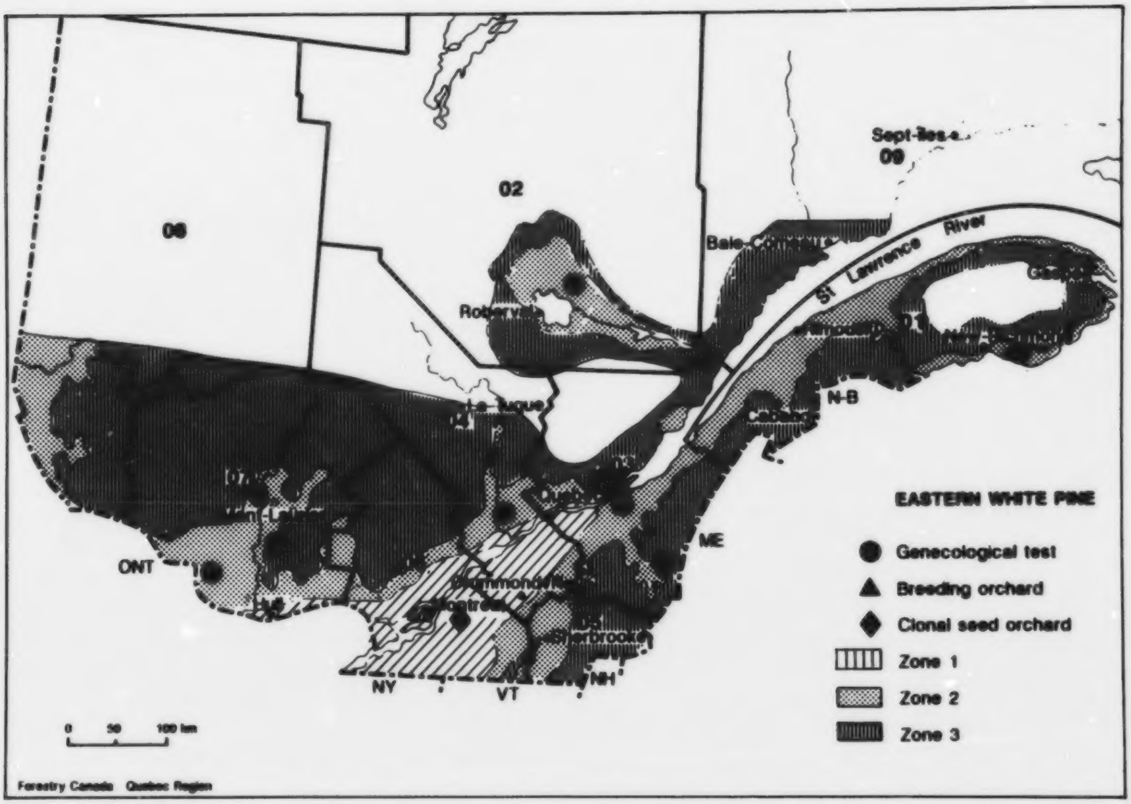
Once the causal agent of these failures was known, it became necessary to look for means of reducing the impact of the problem to a level where the volume of production would still be high enough to ensure reasonable income from the crop. Losses due to white pine blister rust should be under 15%, because other factors such as white pine weevil (*Pissodes strobi* Peck) will also contribute to the reduction of eastern white pine density in the plantation.

A large program of eradication of *Ribes* species was known to have led to beneficial results in the United States (Riley 1930). Such a program has been tried in a much smaller scale in Quebec. In 1931, Pomerleau (1931) established a protection zone around the Berthierville Tree Nursery. Eradication was repeated in 1935 and 1938. Surveys of the infected trees in 1968 showed that *Ribes* control near this eastern white pine plantation maintained the proportion of the rust-infected trees to about 5% (Pomerleau and Bard 1969). In a 25-year-old plantation (Lanoraie) near the site of eradication and where control of the alternate host was not performed, 12.9% of the trees were affected by the disease. Two reasons were put forward by the authors to explain the relatively low level of infection observed in the control plantation at Lanoraie. First, even if no *Ribes* eradication had been done around the plantation, the density of the intermediate host was considered low. Secondly, the possibility that the microclimate prevailing in this area was not favorable to rust infection could not be ruled out. The results of this small-scale program clearly indicated that it was possible to have a productive white pine plantation where the site was appropriate or when the *Ribes* population was controlled.

Based on such results, Pomerleau and Bard (1969) raised the question whether it was still justified to restrict the plantation of a species known for its excellent wood quality, its rapid growth, and its wide adaptation to various types of soil (Zoltowski 1972). The reopening of this issue was even more justified when lumber worth was considered: eastern white pine was worth twice the price of spruce. Pomerleau and Bard (1969) also argued that, despite the presence of this pathogen over the whole range of eastern white pine, the natural populations did not seem more severely affected than previously reported in 1932 (Pomerleau 1932). It was also evident that some protection of eastern white pine was possible by simple silvicultural practices. Therefore, it was recommended that studies of the silvicultural and pathological aspects of eastern white pine production be conducted before definitively rejecting it from reforestation programs.

A reevaluation of the incidence of white pine blister rust in Quebec has been done by Lavallée (1974). He proposed subdividing the range of the species within the province into host-susceptibility zones as a guide to reforestation strategy. Such an approach had been successful in Wisconsin (Anderson 1973) and in several other parts of northeastern North America. Based on the knowledge of the environmental conditions required for the successful blister rust infection of *P. strobus* and on elevation and climatic data, Lavallée (1974) delineated four zones of rust susceptibility: in Zone 1, less than 5% of eastern white pine stems are affected; in Zone 2, the percentage of infected trees is less than 15%; while Zones 3 and 4 present higher levels of risk of infection by *C. ribicola*. The validity of the proposed approach for the area under study was confirmed by the analysis of data recorded over 11 years of observations (Lavallée 1986). Few changes were made to the delineation of the susceptibility zones proposed in 1974, the main correction being the addition to Zone 3 of the area classified previously as Zone 4 (Fig. 1). This zoning indicates that plantation of eastern white pine should be restricted to Zones 1 and 2 where the infection hazard should reach less than 15%. In these areas and on adequate sites, eastern white pine would represent a good investment.

Interest in eastern white pine was then revitalized. Following the publication of the rust susceptibility zoning of the Quebec area, a breeding program taking ecological features into account was proposed by Corriveau and Lamontagne (1977) (respectively, forest geneticist with Forestry Canada in Quebec and tree breeder at the Quebec Ministry of Energy and Resources). As the need for selecting the best genetic stocks for maximium returns from future plantations was of prime importance, the purposes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Sept-Îles
Baie-Comeau
Roberval
St Lawrence River
La Tuque
Québec
N-B
ME
ONT
Drummondville
Montréal
Sherbrooke
NY
VT
NH
EASTERN WHITE PINE
● Genecological test
▲ Breeding orchard
◆ Clonal seed orchard
Zone 1
Zone 2
Zone 3
0 50 100 km
Forestry Canada Quebec Region

Figure 1. Blister rust hazard zones and location of genetic tests and orchards of eastern white pine (*Pinus strobus*) in Quebec.

of the breeding program were 1) to characterize the genetic variability, winter hardiness, and disease resistance in the natural forest of different regions in the province; 2) to select superior genotypes well-adapted to conditions prevailing in the sites of production; and 3) to develop new varieties with improved growth and form. This renewed interest in eastern white pine has also extended to the reforestation program; this species is now part of the reforestation program to a greater extent than before 1978 (Table 1). The province has produced in 1988 nearly 5 million seedlings to meet the requirements.

PRESENT STATUS OF THE EASTERN WHITE PINE COMBINED GENETIC RESEARCH AND BREEDING PROGRAM

Since 1976, 150 populations of white pine in Quebec have been sampled. From these, about 500 seed lots from selected superior phenotypes in natural eastern white pine stands have been collected. Exchange of germplasms with North American and overseas collaborators has contributed to the increase of our gene pool to about 900 seed lots of distinct origins and representing five pine species. A total of 315 provenances and progeny of *Pinus griffithii*, *P. koraiensis*, *P. peuce*, and *P. sibirica* constitutes the exotic gene pool.

Table 1. Number of eastern white pine seedlings planted on private and public lands from 1970 to 1987 in the province of Quebec[a]

Year	No. of plants (thousands)
1970	0.0
1971	0.0
1972	50.0
1973	143.3
1974	153.6
1975	139.2
1976	184.0
1977	24.0
1978	1479.7
1979	340.4
1980	1133.8
1981	1167.5
1982	469.0
1983	295.5
1984	629.5
1985	1110.4
1986	1603.4
1987	566.5

[a] Data obtained from the Reforestation Service, Ministry of Energy and Resources, Quebec.

Superior phenotypes have been selected in five target populations (Lac des Araignées, Lac Brome, Lac Balsam, and the Schyan and Ste-Madeleine rivers) and vegetatively propagated through grafting. These selected phenotypes are maintained in the breeding orchard located at Cap Tourmente near Quebec City.

The Quebec Ministry of Energy and Resources has planned the establishment of 37.5 ha of eastern white pine clonal seed orchard. To date, 28.5 ha have been established located in Regions 03 (Aubin de L'Isle), 06 (Beloeil), and 07 (Egan and Huddersfield) (Fig. 1).

Two phases of a genecological test involving 450 progenies derived from 175 populations have been conducted under intensive culture in nursery by the Laurentian Forestry Centre (LFC). Replications of this genecological test have been established under intolerant forest cover conditions. Seedlings have been planted in strips (2 or 3 m wide) opened through stands of trembling and largetooth aspens and paper birch with few conifers. Five tests have been established since 1986 under forest cover and two in mixture with *Alnus incana*, all in sites located in Zone 2 of susceptibility. Two sites (Fort Coulonge in the Outaouais region, and St-Elzéar-de-Bonaventure in the Gaspé peninsula) were planted in 1986. Three additional localities (Notre-Dame-du-Laus in the Outaouais region, Grand-mère in the Mauricie region, and Notre-Dame-du-Rosaire in the Lake Saint-Jean region) were chosen for establishment of the second phase of the genecological test and were planted in 1988. The two trials involving eastern white pine and alder were established in 1989 at Valcartier and Lévis in an attempt to control the white pine weevil attack.

An early evaluation of the growth and phenology of these progenies indicated no gradient of geographic origin. Studies of the frost tolerance of provenances from a north-south axis suggested that it should be possible to use superior southern sources under our conditions. Some increase of our productivity would by then be achieved (Corriveau et al. 1986).

A total of 160 progenies from 40 populations of exotic pine species have been cultivated under controlled conditions and transplanted in the nursery. This material is being evaluated for winter hardiness, disease (blister rust and scleroderris canker) resistance or tolerance, and white pine weevil tolerance. It constitutes our alien gene pool for the interspecific gene transfer program.

EVOLUTION OF BLISTER RUST IN QUEBEC AND SILVICULTURAL PRACTICES

Studies on the incidence, progress, and impact of the disease on the growth and production of young eastern white pine in plantations are also conducted at the LFC by A. Lavallée. This information will be useful in selecting sites for future eastern white pine plantations with rapid growth and low rust incidence. The aim of this research is also to indicate and identify which types of treatments should be used to lower the level of blister rust infection and to determine the most appropriate time of treatments. Results of part of this research are presented in these proceedings (Lavallée 1990).

FUTURE RESEARCH

Our conventional breeding program will concentrate on breeding and selecting for growth and form. Genetic studies will be conducted using the superior phenotypes selected in the five target populations described above. Research on the inducement of flowering and seed production on young plants through the use of phytohormones, environmental stresses, or other factors will be conducted. This technique will lead to shortening generations and accelerating genetic improvement by earlier testing and selection of breeding stock. Experiments to optimize such treatments will be part of our interpopulation hybridization program. This technique should also ensure a good biennial flower production.

Biochemical studies such as isoenzyme analyses are being undertaken to help define population structures and seed movement criteria. These techniques will also be analyzed as a potential way of detecting different degrees of blister rust resistance and used as a selection tool in an intra- and interspecific hybridization program.

Species hybridization and testing for resistance will be conducted separately from breeding for growth and form. We consider that the genetic variability for blister rust resistance in the species *Pinus strobus* is too low to expect substantial gain through intraspecific breeding and selection. Therefore, we intend to transfer blister rust resistance found in other pine species to *P. strobus* (Hoff et al. 1980). To do so, we will 1) study the mechanisms of resistance and the genetics of these mechanisms, 2) develop a reliable selection system either *in vitro* or by biochemical analysis, and 3) transfer the resistance gene(s) through conventional hybridization or biotechnology. Tissue culture systems will be developed to provide the necessary support to achieve these goals.

Tissue culture and root promotion on physiologically mature cuttings will also become important fields of research designed to make the most of superior hybrids and improved lines.

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RATING A LODGEPOLE PINE FOREST FOR POTENTIAL LOSSES TO COMANDRA BLISTER RUST

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A series of cooperative studies has been conducted by the USDA Forest Service (Rocky Mountain Station, Rocky Mountain Region) and Colorado State University on the biology of comandra blister rust (*Cronartium comandrae* Pk.) and the damage it causes to lodgepole pine (*Pinus contorta* Dougl.) in the central Rocky Mountains (Geils 1984; Zentz 1987; Boyd 1989). Boyd and Jacobi (1989) described the preliminary results of the latest of these studies which examined the frequency and distribution of comandra blister rust on several forest districts. They found a high incidence of comandra blister rust on the Laramie District, Medicine Bow National Forest in southeast Wyoming (USA) that raised concern over the long-term productivity of this lodgepole pine forest for timber production.

We outline here a general method for evaluating the impact of comandra blister rust on the timber productivity of a forest district by developing a hazard rating system. To demonstrate that rating system for the Laramie District, we use the results of previous studies (Geils 1984; Zentz 1987; Boyd 1989) to classify forest sites by predicted stand volume at rotation. Our classification method requires four steps: 1) construct an empirical model to predict timber yields for rust-infested stands, 2) simulate yields for stands representing various combinations of site productivity and rust incidence, 3) identify acceptable levels of timber yield, and 4) identify by site index and distance from the alternate host those stands where timber management and disease control are justified. Although we illustrate these steps for the Laramie District, the method can be adapted to rate the hazard that comandra blister rust poses to timber management on other forests.

MODELING TIMBER YIELD OF INFESTED STANDS

Our best approximation of stand growth and disease development on the Laramie District is expressed as a series of functions in a simple model of timber yield. The purpose of this model is to estimate total cubic volume per unit area at age 100, given only basal area at age 20, site productivity class, and distance of the stand from the nearest population of comandra (*Comandra umbellata* (L.) Nutt.). Site productivity is represented by site index, as estimated from the potential height at age 100 of a dominant or codominant tree that has not lost terminal growth to damage, disease, or severe competition. The functions represent the processes of tree growth, mortality, and disease development for nine cohorts of trees--those never infected and those initially infected in decades two through nine. The model is executed as SPSS/PC+ commands (Norusis 1988) to update average diameter, average height, and number of live trees in each cohort at 10-year intervals and to compute the average canker height for newly infected trees. Cubic volume for all live trees at the end of each decade is the total volume for infected and noninfected live trees.

The model includes relationships to predict: 1) percent of trees infected per decade, 2) canker height, 3) time from infection to top kill, 4) mortality rate, 5) diameter growth rate, 6) height growth rate, and 7) total sound volume. Each relationship can be described by listing the independent variables and describing the mathematical function used for predicting the dependent variable.

Predicted infection rates are derived from the analysis of the temporal and spatial distributions of rust cankers on the Laramie District (Boyd 1989). These analyses suggest that the production of inoculum on comandra and conditions suitable for infection of the pine host are constant from decade to decade. The spatial analysis indicates that the percent of trees infected each decade and subsequently cankered on the main stem is related to: 1) distance from the stand to the nearest population of comandra, and 2) the average diameter of trees in the stand. Infection rate decreases as a negative exponential function of distance and increases linearly with average diameter. Infection rate for each decade determines the number of noninfected trees that become infected and subsequently cankered in the stem.

The average height of stem cankers and time from infection to top kill are modeled from functions described by Geils and Jacobi (1989). The height of cankers that eventually reach and girdle the main stem is a linear function of total tree height at the time of infection. As represented by the model, a cankered stem becomes girdled (encircled by the canker) during the decade following infection; if a recently girdled tree does not die during that decade, it becomes a top-killed tree (dead above the canker and live below the canker).

Mortality rate is computed differently for nongirdled trees, trees infected for one decade (recently girdled), and trees girdled for more than one decade (top killed). The mortality rate for nongirdled trees (noninfected or infected for less than one decade) is adapted from the RMYLD program for generating empirical yield tables of lodgepole pine (Edminster 1978); the competition-related mortality of these trees is calculated by a nonlinear function of the total number of trees per unit area and total basal area per unit area. The mortality rate for girdled trees is a linear function of average canker height for the first decade (Geils 1984) and a nonlinear function for following decades (Geils, unpublished). The 10-year mortality rate of top-killed trees decreases steeply from 65% for trees cankered at a height of 1 m to 10% for trees cankered at 5 m; the mortality rate is nearly constant (5% to 10%) for trees cankered above 5 m.

Diameter growth rates are computed separately for nongirdled trees and top-killed trees. Ten-year diameter increments for trees not infected by comandra blister rust, or those infected for less than one decade, are a function of initial diameter, site index, and total stand basal area (Edminster 1978). For top-killed trees, this increment is proportionally reduced by a factor related to canker height and tree height at the time of infection (Geils, unpublished). As a larger fraction of the crown is lost to top kill, diameter increment is correspondingly reduced. Growth reduction is slight for trees with a canker high in the crown; however, it may exceed 75% for trees cankered low on the stem.

Height growth rates are estimated separately for nongirdled trees and top-killed trees. Ten-year height increments for trees not infected by comandra blister rust, or those infected for less than one decade, are a function of tree age and site index (Edminster 1978). After one decade, infected trees are girdled and height growth stops (Geils and Jacobi 1989).

The volume for each cohort of noninfected trees or trees infected in the same decade is computed with equations provided by Edminster (1978) and modified by Geils (unpublished) for top-killed trees. Total cubic volume of nongirdled trees is determined by average diameter, average height, and number of trees. Total cubic volume of top-killed trees is reduced by a factor that represents the fraction of the total volume that is above the canker height and thus has no timber value. This factor is an exponential function of the ratio of total stem length and canker height.

SIMULATING EFFECTS OF DENSITY, PRODUCTIVITY, AND DISEASE SEVERITY

The second stage of our methodology for hazard rating is to simulate the development of managed stands that represent the range of productivity and disease incidence levels that occur on a 120 000 ha block of the Laramie District. These simulations indicate the expected volumes if stands were naturally regenerated to lodgepole pine, thinned at age 20, and clearcut at age 100. For this exercise, we include two levels of site productivity--site index 12 m (the minimum for commercial stands) and site index 15 m (the most productive sites on the district). Management options allow for thinning at age 20 to either 860 trees per hectare (minimum acceptable stocking) or to 1240 trees per hectare (maximum recommended stocking). Precommercial thinning of naturally regenerated lodgepole pine is commonly used on this district to reduce density and improve spacing. Variations in disease severity are generated by distances to the nearest comandra population from 0 km (at the forest edge) to 8 km. At age 100, about 40% of the lodgepole pine in stands near the forest edge are cankered by comandra blister rust; incidence drops to about 5% at a distance of 8 km (Boyd 1989).

Analysis of these simulations suggests that timber yield at age 100 is influenced strongly by site index and distance from comandra, and only slightly by post-thinning density. Projections show only a 10% increase in final volume for stands initially stocked with 1240 trees per hectare in comparison to stands with 860 trees per hectare. For example, a stand that is 4 km from the nearest population of comandra with a site index of 12 m is expected to yield 140 m^3/ha if stocking at age 20 were 860 trees per hectare and only 160 m^3/ha if stocking were increased to 1240 trees per hectare. In contrast, an increase in site index from 12 m to 15 m nearly doubles the yield; an increase in distance from 0 km to 8 km from the nearest population of comandra also doubles the yield (Fig. 1).

Incidence levels and volume reductions are small enough at a distance of 8 km that yields from these stands serve as a reference for volumes in the absence of rust (potential volume). Relative volume loss caused by comandra blister rust is defined as the percentage reduction from potential volume. Relative volume loss increases sharply (0% to 70%) as distance from comandra decreases from 8 km to 0 km (Fig. 2).

DEFINING MANAGEMENT CRITERIA AND HAZARD CLASSES

The third stage in our rating methodology is to define several hazard classes by criteria that are relevant to timber managers. The first criterion is that a stand be capable of producing a sufficient volume to justify an investment in timber management, for example, the costs of regulating and regenerating the stand. On the Laramie District, sites incapable of producing at least 105 m^3/ha at age 100 are poor candidates for an emphasis on timber management. The second criterion is that the disease threat must be large enough to warrant special actions to mitigate losses. According to district forest managers, disease losses are sufficient for additional stand entries (sanitation or salvage) if potential yield were reduced by more than 15%.

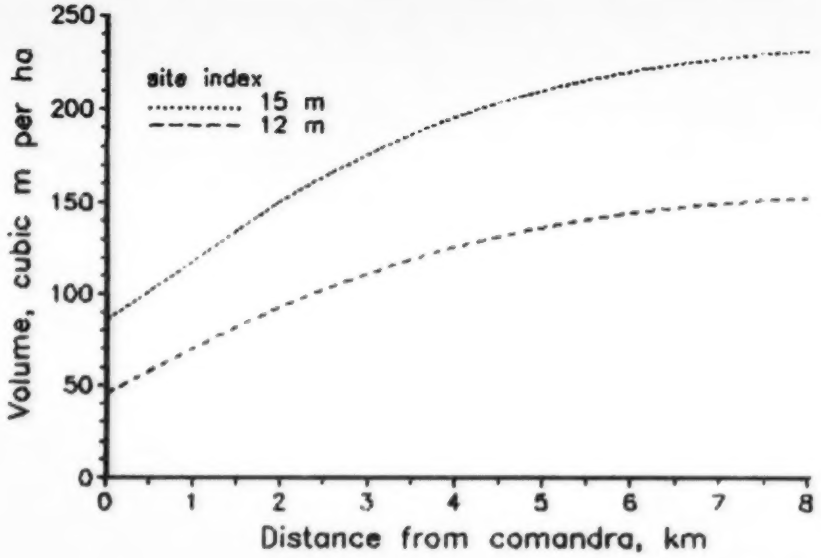

Figure 1. Total volume of lodgepole pine stands at age 100 by site index class and distance from the nearest population of comandra.

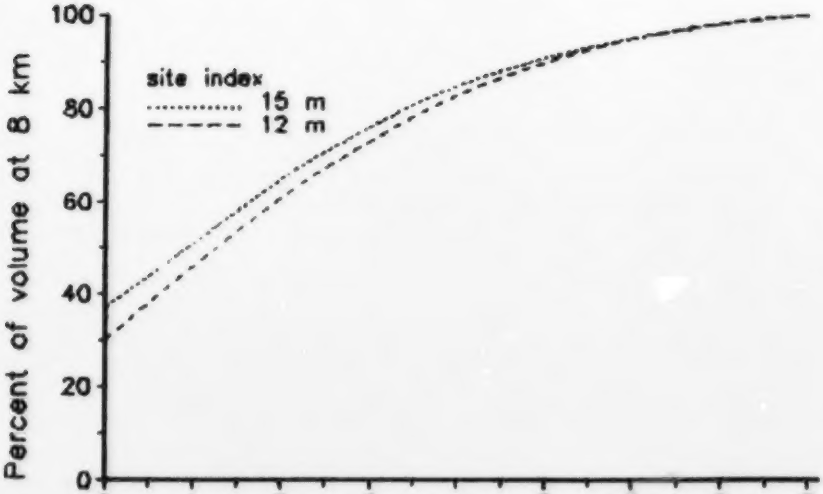

Figure 2. Percent of potential volume yield for lodgepole pine stands within 8 km of the nearest population of comandra.

Applying these resource-specific criteria, we define three hazard classes. Sites incapable of producing at least 105 m^3/ha because of low site productivity and severe rust damage have a high hazard rating. Sites that produce more than 105 m^3/ha but lose more than 15% of their potential volume because of disease have a moderate hazard rating. Sites capable of producing both more than 105 m^3/ha and more than 85% of their potential volume have a low hazard rating.

IDENTIFYING HAZARD BY SITE INDEX AND DISTANCE FROM COMANDRA

The hazard posed by comandra blister rust at any location, as characterized by site index and distance from the nearest population of comandra, is determined by comparing the predicted volume and volume loss from the yield model (Fig. 1, 2) with the selected management criteria. Such comparisons show that good sites (site index 15 m) within 0.5 km of a population of comandra have a high hazard rating; fair sites (site index 12 m) within 2.5 km have a high rating. Stands with a high hazard rating (about 5% of the 120 000-ha district) are not expected to produce sufficient yields (105 m^3/ha) to justify investments in timber management. Rust hazard is moderate for all sites capable of producing >105 m^3/ha, but closer than 4.5 km to the alternate host (about 15% of the district). These are the stands where potential values and expected losses are large enough that special disease control activities (sanitation and salvage) should be considered. Rust hazard is low on sites capable of producing >105 m^3/ha and further than 4.5 km from a comandra population (about 80% of the district); on these sites, the small losses caused by comandra blister rust may be further minimized by normal silvicultural practices.

APPLICATION AND CONCLUSION

For a hazard rating system to benefit forest managers, it should use available site attributes and describe levels of disease severity in appropriate terms for the threatened resource. In this example, we used site index and distance from the nearest population of comandra to predict expected volume yields following a simple, but reasonable management scenario. Because the distribution of the alternate host along the eastern flank and in the southwestern corner of the district is known (Boyd 1989), distance from any stand to the nearest population of comandra can be measured on a map or computed by a geographic information system.

By expressing disease severity as a proportion of resource lost, rather than as a percent of trees cankered, the forest manager can directly interpret the importance of comandra blister rust on any site. In spite of the low level of timber productivity on this district, volume realized at rotation age is still a good index for the hazard rating system. Expressed as total yield and percent loss, volume estimates are useful for evaluating numerous management alternatives, including disease control to minimize impacts from comandra blister rust.

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